

A good week for the world

It would be hard not to be optimistic after last week's progress on arms control. But the next step will require some much more fundamental changes in thinking.

THIS has been a busy week for negotiators around the world, but especially so for those in the United States and the Soviet Union. In Montreal, representatives of 24 nations hammered out a treaty that will limit the use of chlorofluorocarbons, increasingly implicated as playing a critical role in the complex chemistry of the upper atmosphere (see page 277). Later in the week, the Kremlin and the White House announced to the world that the basis for a treaty on intermediate nuclear weapons had been agreed upon, the details of which would be cleared up before a summit between Mr Reagan and Mr Gorbachev takes place sometime before the end of the year.

Negotiating the treaty, difficult as it may be, is only one of an interlocking series of moves that is required if the world is to change the way it conducts its business. Even as President Reagan proudly announced his arms-control achievements, the Senate voted to curtail certain experiments contemplated by the Pentagon as part of the president's Strategic Defense Initiative (SDI) because they would violate the antiballistic missile (ABM) treaty. The Reagan administration has sought to show that SDI and ABM are not incompatible acronyms, but even conservative Congressmen find it hard to stretch conscientiously the language of the latter to include all the activities required by the former.

Negotiators in Montreal can be justifiably proud of their accomplishments. Although it would be pleasing to have clear-cut answers about how chlorofluorocarbons affect ozone concentrations in the atmosphere, the certainty that there is now no way to coax them out of the atmosphere once they are released into it must certainly make countries consider the implications of using them, especially now that the link to adverse consequences can be reasonably postulated. Although some will argue that the restrictions on the use of chlorofluorocarbons have not gone far enough, the very fact that restrictions went beyond what seemed possible a few years ago should make those desiring greater reductions confident that they are not being ignored. This is a time to treat the world's cup of environmental consciousness as half-full, rather than half-empty.

Defiance

Similarly, it is possible to look critically at what was not accomplished between the Soviet Union and the United States in their new agreement without losing a sense of optimism. The Soviet Union would like to see ABM strengthened, with the intention of putting an end to SDI. The United States does not find that appealing. Indeed, at the end of the week Secretary of Defense Caspar Weinberger gave formal approval to a series of SDI tests that — while staying within the currently accepted boundaries of ABM — must seem defiant given the spirit of agreement in the air in Washington. There is also little more than hopeful noises of progress toward an agreement reducing numbers of long-range strategic weapons. Even the current agreement on short- and intermediate-range missiles has problems that must be solved regarding timetables and verification.

But much has been achieved and an agreement, even if it is not complete, can engender the good will that is necessary but not sufficient for further agreement. The United States will

eliminate its ground-launched cruise missiles deployed throughout Europe and its Pershing 2 missiles currently deployed in West Germany. The Soviet Union will remove its SS-20 and SS-4 intermediate-range weapons and its short-range SS-12 and SS-23 missiles. That the European nations have been persuaded that this arrangement is in their best interests is in itself remarkable, as is the Soviet Union's acceptance that discussion of British and French nuclear forces, which are to all practical purposes the same as the weapons now to be removed, should be put on one side for the time being.

Warming

A flurry of activity may be expected to follow in the wake of the warming of relations between the Soviet Union and the United States. Scientific cooperation is likely to be one beneficiary. Already there is welcome talk of new initiatives in oceanic experiments, in assessing the scope of the depletion of ozone from the atmosphere and in expanding the number of exchanges of scientific personnel that have, under the Reagan administration, become largely a matter of individual initiative. There is even the possibility of some progress in devising a more rational international division of labour in the study of the practical use of nuclear fusion. Progress in these areas provides optimism that the larger differences separating the two superpowers may yet be resolved.

Agreement has come this far without President Reagan having to bite the bullet and make concessions on the timetable for the development and deployment of SDI. But unless the Soviet Union makes a surprising turnabout, progress towards a more complete agreement on long-range nuclear weapons will certainly involve some form of decision not to deploy weapons in space. Reagan's own very human desire to see a strategic arms treaty take shape before the end of his term of office will, paradoxically, be made the harder to realize by his belief that strong support for SDI has helped win the present agreement from the Soviet Union. Nor can SDI be disentangled from another issue, that of nuclear testing, that will almost certainly have to be resolved before agreement can be reached on a wider range of arms-control issues.

The Union of Concerned Scientists estimates that the United States has spent some \$9,000 million building and deploying the weapons it has now agreed to scrap. But even this massive throw away will not compensate for the build-up in the long-range nuclear arsenal that has occurred under Reagan. The move from an agreement on intermediate-range missiles, where both the Soviet Union and the United States could gain clear political advantages in Europe without either having to make concessions that fundamentally threaten their security, to an agreement to reduce the number of long-range nuclear weapons, is a step that to be accomplished successfully will require a massive leap of the imagination. If Reagan is to look back on his period in office as one that brought about decisive progress, he will have to find some way soon to match hints of Soviet flexibility on continued research on SDI with his own hints that the Senate view of the ABM treaty could have something to recommend it. □



Japan's moves support CoCom

- Reagan and Nakasone agree on Toshiba
- Stealthy subs issue submerges
- Communist countries remain sceptical

Tokyo

How much substance is there to the allegation that the illegal export of milling machines to the Soviet Union by Toshiba Machine Co. has resulted in a serious breach in the West's security? As the case unfolds it appears that the Toshiba affair has more to do with the internal politics of the United States and the desire of Prime Minister Yasuhiro Nakasone to strengthen US-Japan military links than to the silencing of Soviet submarines.

Nakasone arrived in the United States this week secure in the knowledge that his actions on at least one trade issue have been warmly welcomed by the administration of US President Ronald Reagan. It is less than five months since Toshiba Machine's illegal export of multi-axis milling machines to the Soviet Union was disclosed during Nakasone's last visit to Washington. But already Japan's laws have been rewritten to clamp down on high-tech exports to communist countries.

Earlier this month, the upper house of the Diet passed a revision of the Foreign Exchange and Foreign Trade Control Law which strengthens fines and sanctions against companies violating the rules of CoCom (the Coordinating Committee on Multilateral Export Controls). The Ministry of International Trade and Industry (MITI) will more than double the number of officials monitoring exports to communist countries, and are implementing strict self-control measures.

US Secretary of Defense Caspar Weinberger praised Japan's quick efforts to strengthen CoCom-related laws when MITI minister Hajime Tamura visited Washington on 10 September. And Weinberger said that it would have taken 15 years to enact such legislation in the United States. Therein may lie the key to the whole Toshiba affair.

Earlier this year the US National Research Council argued in a report that the Pentagon is hurting US industry through limiting exports to the Soviet bloc (*Nature* 325, 291; 1987). And the US Congress has incorporated provisions into its omnibus trade bill to reduce the number of items on the "controlled list" and to loosen export regulations. The Pentagon, on the other hand, is pressing for stricter controls.

The US Defense Department knew about Toshiba Machine's illegal export of milling machines to the Soviet Union long before the story broke in the Japanese and US press in late April and some US obser-

vers suspect that the Pentagon may have used the Toshiba incident to counter the moves by Congress. Although stressing that the Toshiba sale was illegal, William A. Root, former head of the US CoCom delegation, said the issue had been "dramatized" by the various parties, and that the timing looks "particularly motivated".

Central to the Pentagon's condemnation of Toshiba Machine Co. is the claim that the milling machines have been used to make propellers that produce less cavitation noise and, as a result, Soviet submarines are now harder to detect. But the crucial factor determining cavitation noise is the design of the propeller, not the machines that mill it. And there are many other sources of noise in submarines apart from the propeller. Furthermore, quieter Soviet submarines began to appear before the Toshiba machines were operational (*Nature* 328, 283; 1987).

According to Root, illegal exports of

Soviets stress CoCom complications

London

SOVIET political commentators, who to say the least, are naturally not enamoured of CoCom, are presenting the Toshiba affair as an attempt by the United States to keep west Europeans out of Eastern-bloc markets, both for political and commercial motives. This line of argument has been particularly aimed at the Scandinavian audience, in view of the alleged involvement of the Norwegian firm Kongsberg Vapenfabrikk. Moscow radio's Swedish and Finnish services have also linked the Toshiba story with Sweden's adoption last year of new export controls which are basically identical with those of CoCom (unlike Norway, neutral Sweden is not a CoCom member), although the new law was adopted many months before the Toshiba affair became public. They were, in fact, inspired by an attempt to smuggle computer hardware and software to the Soviet Union via Sweden, in 1983 (see *Nature* 303, 635; 1983).

The Soviets present the new law as evidence of US pressure on Sweden, and cite a report in the Stockholm daily *Dagens Nyheter* last month that, at the request of the United States, Swedish customs are now investigating two Swedish companies alleged to have secretly supplied a socialist country with US-made microprocessors.

Vera Rich

high technology to communist countries from western Europe and the United States are common. For example, the Soviets broke off previous negotiations with Toshiba in 1981, claiming they could get the same items at lower prices elsewhere. And, in a recently released report on the Toshiba incident prepared by US and Japanese law firms at the request of Toshiba Corporation (the parent company of Toshiba Machine Co.), it was revealed that Toshiba Machine employees saw a multi-axis propeller milling machine made by the French firm Forest Line when they were installing their machines at a Soviet shipyard on the Baltic in 1983. Forest Line deny breaking CoCom regulations restricting export of machines with more than three axes, but multi-axis machines can be made by running simpler devices in tandem, using control systems of the kind made by Kongsberg-Vapenfabrikk of Norway.

The Toshiba story surfaced in the press at a time that Japan and the United States were finalizing an accord to Japan's participation in the research phase of the US Strategic Defense Initiative (SDI). And the hullabuloo over Toshiba has allowed the Nakasone administration to rush the toughened export control law through the Diet, thereby preparing the stage for the participation of Japanese companies in SDI (according to leaks to Japanese newspapers, the secret SDI accord calls for strict observance of CoCom regulations by both countries). Nakasone has also called for US-Japan cooperation in antisubmarine warfare research in the wake of the Toshiba incident and consultations have already been held.

The Nakasone and Reagan administrations, however, are not alone in taking advantage of the Toshiba incident. US congressmen have gone on a rampage of "Japan bashing" that has included pulverization of Toshiba cassette recorders in public. And Congress has introduced clauses in its trade bill to ban imports of Toshiba products to the United States. But the Reagan administration has vowed to fight these and other protectionist clauses by, if necessary, invoking a presidential veto. US industry has recently rallied to the administration.

The most significant consequence of the Toshiba affair for Japan, however, may be a deterioration in relations with communist countries. China is complaining that Japanese companies have cancelled \$1,800 million in contracts since the Toshiba case. MITI is considering slackening export regulations for China, but for the other 13 communist countries on the ministry's list strict controls will be maintained.

David Swinbanks

Brazil enriches uranium

BRAZIL'S President José Sarney has announced that the country has now "mastered" the ultracentrifuge method of uranium enrichment, and pledged that the technology is to be used for peaceful purposes only. An enrichment plant has been established at the Resende industrial complex of the Nuclebras nuclear corporation. The director of Nuclebras, David Simon, says that in 1989 Brazil will export enriched uranium to Argentina, and that the first consignment will consist of 5 tonnes of uranium enriched to 0.85 per cent. A São Paulo newspaper quotes a Nuclebras official as saying that the president's announcement "fell like a bomb" on Nuclebras, since the latter's management had been "discussing" the alternative, West German, process for years "without technological barriers being overcome".

V.R.

Eureka cash flows

RESEARCH and technology ministers from 20 European countries (including non-EEC members: Scandinavia, Austria, Switzerland and Turkey) met in Madrid last week to consider new projects put forward for funding by the Eureka research incentive programme. This was the fifth meeting since Eureka was set up in 1985 to increase European industrial cooperation in high-risk, new technology research.

The 58 new projects approved at the meeting represent an investment of 709 million European Currency Units (ECU), about £490 million, by member states and will bring the total number of projects supported by Eureka to 165. Priorities agreed in Madrid include: information technology (9 projects, with a grant of 62 million ECU), bioengineering (11 projects, 27 million ECU), energy (3 projects, 25 million ECU) and new materials (2 projects, 10 million ECU).

P.C.

PWR at Hinkley Point

THE application of Britain's Central Electricity Generating Board (CEGB) for the country's second pressurized water reactor (PWR) seems certain to lead to a public inquiry. But the CEGB will not comment on the likely duration of an inquiry into the new Hinkley Point PWR, after the two-year marathon for the PWR at Sizewell B. The CEGB plans to start building the second £1,500-million PWR in 1990. It will cost £200 million less than its Sizewell predecessor.

Local authorities are objecting to Hinkley C. The CEGB proposal calls for the new nuclear reactor to be sited on four hectares of foreshore taken from a national nature reserve.

Electricity planners are considering six other potential sites for PWRs in England and Wales, as part of a programme to reduce dependence on coal-fired power stations.

K.J.

US weapons laboratories an obstacle to treaty?

- Necessity for nuclear tests questioned
- University regents accused of "whitewash"

San Francisco

ARE the Los Alamos and Lawrence Livermore National Laboratories standing in the way of a comprehensive test-ban treaty? A group of University of California physicists think so, and brought their case before the University of Californian Regents at their 17 September meeting in Los Angeles.

The physicists were alarmed by testimony by Roger Batzel, director of Lawrence Livermore Laboratory, to the House Armed Services Committee in September 1985. Batzel said nuclear weapons have been designed under the assumption that testing will never be banned, so continued testing is necessary to maintain their reliability.

The faculty critics oppose a design policy that requires continued nuclear testing. They feel the laboratories have too much autonomy under rather loose management by the University of California, and influence government policy to promote their own interests. These interests include an aversion to test-ban legislation that might put an end to the main livelihood of the laboratories, the development of new weapons.

The laboratories argue that they are following orders from the Department of Energy. But critics cite a potential conflict of interest because the laboratory directors also act as advisors to the government on weapons and arms control.

Lawrence Livermore theoretical physicists Hugh DeWitt and Ray Kidder charge the laboratories with misleading people about the reliability of weapon stockpiles. DeWitt and Kidder believe that current stockpiles can be relied on without nuclear testing, and many prominent physicists, including Glenn Seaborg, head of the Atomic Energy Commission for 10 years, Hans Bethe, former director of the theoretical division at Los Alamos, and Norris Bradbury, former director of Los Alamos, agree with them.

Critics claim that the laboratories have pushed weapon design parameters to their limits, in the interest of maximizing the yield-to-weight ratio. An alternative approach, says DeWitt, would be to build larger and heavier weapons that would be more certain to perform correctly. Ageing weapons could be replaced by remanufacture of identical models. The more forgiving design would ensure adequate performance without repeat nuclear tests.

John Immele, deputy associate director

for nuclear design at Livermore, calls the remanufacturing idea a "pipe dream of theoreticians". Materials change, he says, and there is no way, short of a test, to be sure that remanufactured weapons will fire with the expected yield.

In addition, Immele says a comprehensive test ban would result in a drain of expertise, jeopardizing the remanufacturing process. As experienced weapons experts retired or left, new trainees who had never studied a nuclear blast would be ill-equipped to oversee the successful manufacture of nuclear weapons.

Variations of this debate have been raging for nine years, without much change in the *status quo* at the laboratories. Last year a faculty letter-writing campaign, instigated by Walter Kohn and Jose Fulco of University of California, Santa Barbara, persuaded university president David Gardner to order an inquiry into the faculty concerns.

The results of the inquiry by the Scientific and Academic Advisory Committee (SAAC) that oversees the laboratories, released last July, disappointed laboratory critics, who called it a "whitewash" job that found the laboratories to be "without a single blemish". But to no one's surprise, the regents accepted the SAAC report as fulfilling President Gardner's request and rejected a rebuttal prepared by the faculty group.

The scrutiny of the weapons laboratories does not end here. The university's academic senate recently set up a faculty committee to investigate the laboratories' activities and, at the request of six members of Congress, Kidder has conducted a detailed study of weapons reliability. Batzel allowed Kidder to do his analysis, but the laboratory is to submit an independent study to Congress.

In response to the laboratories' concern that a test ban would cause a drain of expertise, DeWitt and Kidder suggest that a low-threshold test ban, allowing tests of under one kiloton, would allow research and training to continue. Immele disagrees. "One kiloton is the same as zero kilotons", he says, and would allow neither understanding of the physics involved nor certification of reliability. On 19 May the House of Representatives passed a defence bill that would limit nuclear tests to less than one kiloton, if the Soviet Union agrees to do the same. A similar bill will soon be considered in the Senate.

Marcia Barinaga

French jubilant but British equivocal after Ariane success

Paris

THE successful launch of Ariane 3 in the small hours of 16 September, after a 16-month interruption, caused unrepressed jubilation in France, but restrained applause in Britain. This was not simply a question of Latin ebullience and English reserve. Whereas France is committed to a long-term European space programme and stands to profit from Ariane's success, Britain's role is becoming minimal, following government refusals to provide money for a bigger stake (see *Nature* 328, 565; 1987).

Ariane has been out of service since its V18 flight was aborted seconds after lift-off in May 1986, due to ignition problems in the third-stage cryogenic motor. With these problems overcome, Arianespace, the company set up by the European Space Agency (ESA) to market and operate Ariane, becomes the only western company able to offer commercial satellite launches. This lead will only last until 1991, when the backlog of orders, worth £1,500 million, should have been cleared and when other competitors present more of a threat.

The Americans have been out of the running since the Challenger shuttle disaster and problems with their conventional launchers last year. US companies manufacturing Thor Delta and Titan rockets have recently been revived by large orders from the US Air Force and will undoubtedly wish to use the technology to compete in the commercial sector, particularly following the recent successful launches of Chinese, Soviet and Japanese rockets.

To keep its position in space into the next decade, ESA therefore needed to

restore Ariane's credibility as a reliable commercial launcher. This assurance was not so much for Arianespace's customers, whose outstanding orders are already partly paid for, but to convince sceptical ESA members that extension of Europe's space programme is not throwing good money after bad. At a crucial ESA ministerial meeting to be held in The Hague in November, members will be asked to commit themselves to Europe's 'Columbus' contribution to the proposed \$18,000 million US National Aeronautics and Space Administration (NASA) space station (see *Nature* 329, 4; 1987).

With a 60 per cent stake in Arianespace, compared to Britain's 2.4 per cent, France has the most to lose if Columbus does not get the necessary backing. Part of the Columbus programme, the next generation of Ariane 5 rockets would be used to launch the controversial French Hermes shuttle.

A shadow was cast on plans for the Columbus programme to link up with the NASA space station when ESA and NASA failed to agree on fundamental terms of reference at a meeting in Frascati, Italy last week. The United States, the major financial backers, want overall control of the station and an option to use it for military research, both vigorously contested by ESA delegates.

Mrs Thatcher's unwillingness to support the £200-million British National Space Centre (see right) has also provoked questions in ESA regarding Britain's commitment. With Ariane's success there is now a growing feeling that Britain's contribution, while welcome, is not essential.

Peter Coles

UK industry's late space bid

London

WITH barely 6 weeks remaining before ministers from the 13 member states of the European Space Agency (ESA) meet in The Hague to decide on the next suite of European space programmes, Britain's commercial space community is making last-ditch attempts to persuade the government to reverse its decision not to increase investment in space research. Hopes are being pinned on the government's newly formed Advisory Council on Science and Technology (ACOST), which will meet on 29 September. One of ACOST's functions is to advise the government on "the nature and extent of United Kingdom participation in international collaboration in science and technology".

The ESA summit is expected to ratify three main programmes over the next decade, needing at least a doubling of the present annual budget of £1,000 million. Because of ESA's strict industrial returns rule, member states receive contracts in proportion to their contribution to the budget. Britain has a 12 per cent stake in ESA. In July the prime minister, Mrs Margaret Thatcher, announced that the government's £112-million space budget would not increase "for the time being".

Last week, Britain's industrial space community stepped into the fray with the formation of a new company called Space Ventures Ltd, whose main objective "the promotion, for the benefit of the United Kingdom, of the opportunities arising from the exploitation of space".

Initially the company is being supported by British Aerospace, Marconi Space Systems, Logica, Westland Aerospace and Smith Associates. The company proposes to act as a link between technology suppliers, the government's British National Space Centre and financial institutions. The organizers will try to show the government that industry can provide more support for national space programmes.

In addition, the UK Industrial Space Committee has produced a booklet entitled *British Industry in Space—a Time for Decision*, which emphasizes the importance of the economy of public-funded space research, and says that for Britain to maintain its current position within ESA, national expenditure would need to be increased to £250 million annually over the next 3 years—less than 4 per cent of the government's research and development budget.

Opinion is divided over whether, after the ACOST meeting, any extra funds will appear and, if so, whether it will be too soon before the ESA meeting on 9 and 10 November to make any difference.

Simon Hadlington

Indian satellite failure still a puzzle

New Delhi

A FIVE-MONTH investigation by a committee of experts has failed to pinpoint the cause for the failure of India's Augmented Satellite Launch Vehicle (ASLV) that crashed during its maiden launch on 24 March. The committee confirmed that the first-stage motor failed to ignite 48.5 seconds after lift-off, but could not identify the cause of the malfunction.

The Indian space research organization (ISRO) said that 37 possible reasons for the failure were identified, but detailed investigation showed that none of those were responsible. In the absence of a definite clue, it has been assumed that ignition failure could have been caused by a short or open circuit of the ignition system, or by random malfunction of the safe-arm device. ISRO says reliability of these three

components will be improved in the next ASLV scheduled for February 1988, carrying a payload from West Germany.

Meanwhile, the successful launch of Ariane on 16 September has brought relief to ISRO, which had been worried about placing the Insat-1C communications satellite as a replacement for Insat-1B, early in 1989. The meteorological, telecommunications and television services in India critically depend on the Insat system and ISRO is confident that Insat-1C will be launched by Ariane on schedule in June 1988. Another project scheduled for next year is the Soviet launch of the Indian-made remote sensing satellite (IRS-A) for which ISRO has agreed to pay 75 million rupees (\$6 million) for launch costs. The Soviet Union has in the past launched three Indian satellites free of cost. K. S. Jayaraman

Looking ahead to the greenhouse after ozone agreement reached

London

LAST week's international agreement to protect the Earth's ozone layer from destruction by man-made chemicals (see *Nature* 329, 189; 1987) may spur policy-makers to take on the complex issue of the greenhouse effect. Chlorofluorocarbons (CFCs), regulated by the new agreement because of links with ozone damage, are also 'greenhouse' gases, absorbing infrared radiation. The resultant warming effect is predicted to cause worldwide changes in temperature and weather patterns, as well as a substantial rise in sea level.

The United Nations Environment Programme (UNEP), which led international efforts to protect the ozone shield, is also studying ways of reducing emissions of greenhouse gases, and its director, Mostafa Tolba, has been asked by UNEP's governing council to come up with policy options by next year. The US Environmental Protection Agency is also studying the problem.

Controlling the greenhouse effect is far more complicated than implementing controls on CFCs. Other greenhouse gases are related to a complex web of energy policies, air pollution and other human activities.

The need to tackle the problem of the greenhouse effect was referred to at last week's Montreal meeting of signatories to the Vienna Convention for the Protection of the Ozone Layer, where 24 countries signed a protocol limiting the

production and consumption of CFCs and halons. The final agreement included compromises to lessen the effect on developing nations which want to continue to use CFCs as refrigerants — the lid on CFC production is 10 to 15 per cent higher than the cap on consumption to allow exports from producer countries.

Another compromise would allow the Soviet Union to complete two CFC plants now under construction, and to increase its per capita consumption. Even so, the Soviet Union did not sign the protocol, but said it may do so later. The final agreement calls for a 50 per cent cutback in consumption of fully halogenated CFCs by 1999, with a 20 per cent cutback in 1994 and a freeze in 1990. Countries representing two-thirds of consumption must ratify the protocol before it comes into force.

The chemical industry is working on recovery and recycling processes for CFCs, but the race is also on to develop alternatives, a process that industry representatives say will take 5–10 years. Some alternatives already exist. For refrigerators and automobile air conditioners, CFC 22, which is largely destroyed in the lower atmosphere because it contains hydrogen, could be substituted for ozone-damaging CFC 12 with some re-design of equipment. Fluorocarbon 134a contains no chlorine and therefore cannot destroy ozone, and is thought to be the most attractive long-term substitute.

Kathy Johnston

Hackers breach computer security across three continents

Munich

RESEARCH organizations throughout the world were busy last week playing down the importance of a penetration of their computer systems by an anonymous West German group. A total of 135 computer systems in Europe, the United States and Asia, including those of the US National Aeronautics and Space Administration (NASA), CERN, ESA, the German Aerospace Research Establishment and the European Molecular Biology Laboratory (EMBL) were penetrated through the computer network SPAN (Space Physics Analysis Network).

Chris Sander of EMBL says there is "no visible damage" to the EMBL computer system. EMBL already guards its research computers and may switch its administrative accounts onto a new system, a move it "would have done anyway", says Sander.

NASA says that no confidential data

were compromised, despite the claim by the intruders that they gathered 200 pages of sensitive documentation before the alarm went out about the breach in August. Several NASA drawings (of the space shuttle, for example) were shown on West German television on 15 September and cited as proof of the break-in.

The computer 'hackers', speaking through the Chaos computer club of Hamburg, West Germany (which takes no responsibility for the break-ins), said they intended no harm and that the public announcement of their success was meant as a "warning" to the computer manufacturers and system operators that their machines are not secure. The hackers first accidentally entered SPAN in the spring of this year by falling through a 'loophole' in the software. They left a 'Trojan horse' inside the system, a program enabling them to re-enter at will.

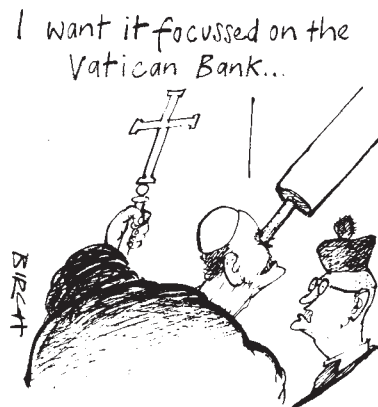
The presence of the intruders was

Vatican astronomers are visited by Pope

Phoenix, Arizona

DURING his recent US tour, Pope John Paul II took a few minutes out from his busy schedule for a lesson in telescope making. While in Phoenix, Arizona, the pontiff spent 15 minutes in a private session with astronomers from the University of Arizona, the Vatican Observatory and the National Optical Astronomy Observatories, arranged by George Coyne, director of the Vatican Observatory and adjunct professor at the university.

Since 1981, Vatican astronomers have spent about 9 months a year studying stars from the university's Steward Observatory in Tucson. The group hopes soon to have its own 1.8-metre Vatican Advanced



Technology Telescope based in Arizona. This telescope will be the first produced by spin-cast technology, a process for making honeycomb mirrors with very short focal lengths. It was developed by J. Roger Angel at the University of Arizona.

The Pope is not an astronomy buff, said Boyle, but is "very seriously interested in science and religion and in the relationship between science and religion."

The Vatican Observatory is the Vatican's only formal research institution in the physical sciences. It was set up in 1891 by Pope Leo XIII, at Castel Gandolfo, the papal summer residence near Rome. Light pollution prompted Vatican astronomers to seek darker skies in Arizona.

Elizabeth Pennisi

discovered in early August by a systems manager at EMBL, who alerted other users. No criminal proceedings are expected. Legal provisions do exist but there are few precedents, and "evidence would be extremely hard to come by", said Steffen Wernery of Chaos.

Changing operating systems and installing more security measures in SPAN might provide temporary relief to the legal users. But all agree that security problems are here to stay. Said Wernery, "The hackers always try to stay at the cutting edge."

Steven Dickman

BNFL and Nirex in deep water over nuclear waste disposal plans

London

PROPOSALS by British Nuclear Fuels Ltd (BNFL) to bury intermediate-level waste deep under the Irish Sea near Sellafield have incensed the Irish government and undermined plans by the British radioactive waste agency Nirex.

BNFL made the surprise announcement of its investigations into deep-sea dumping as part of a press release on its plans to spend £20 million to improve its low-level nuclear waste disposal site at Drigg. Conceptual plans call for a repository 800 m below the seabed, half a kilometre offshore.

This is an embarrassment for Nirex, which is responsible for the development and operation of low and intermediate level waste facilities. It had just announced that public consultation would be sought on possible burial methods for nuclear waste through a consultative document to be published in November.

The Nirex move is perceived as a way to overcome public criticism and the so-called Nimby (not in my back yard) syndrome, as well as accusations that the government's nuclear waste disposal policy has been in a state of confusion ever since the pre-election abandonment of four potential shallow waste disposal sites in marginal Conservative seats.

Although Nirex is responsible for nuclear waste disposal, BNFL, which has a 40 per cent share and directors on the board of Nirex, apparently developed its plans without consulting the rest of the industry.

Deep burial in the seabed close to Sellafield is seen by BNFL as preferable to transporting the waste. Sellafield, which reprocesses nuclear fuel from several

European countries and Japan as well as Britain, produces two-thirds of Britain's intermediate-level nuclear waste. An estimated 180,000 m³ of intermediate-level waste will require disposal by 2030, and that would increase if the government dismantles its ageing Magnox reactors.

In contrast to the strident public opposition to Nirex's plans for shallow disposal, BNFL is not expecting much local opposition in Sellafield, where many residents are employed in the industry. But Ireland has pledged to put pressure on the British government to veto the seabed plan.

Nirex has already called for pre-bids from companies interested in designing and building a deep depository. The agency's consultative document will call for public submissions on three options: a deep bore-hole on the mainland with horizontal shaft connecting to disposal galleries under the sea; a shallow rail tunnel leading to undersea disposal galleries; or offshore boreholes deep into the seabed.

Kathy Johnston

Medical research on the block

London

IN the continuing campaign to persuade the government to reverse its declining investment in scientific research, the heads of Britain's universities last week sought to focus attention on the mounting pressures facing clinical academic medicine.

At a meeting attended by the higher education minister, Robert Jackson, the Committee of Vice-Chancellors and Principals (CVCP) warned that falling numbers of university-funded clinical academic staff and increasing dependence on non-government-funded research contracts is causing "extreme concern" within the medical community. Between 1981 and 1986, the number of full-time clinical academic staff funded wholly from university sources fell by 13.4 per cent to 2,176. During the same period, the number of clinical academic staff funded from other sources, increased by 91 per cent to 1,668. While the government sees this as a vindication of its insistence that more money is available from the private sector, academics are becoming increasingly worried about the implications.

According to CVCP chairman Sir Mark Richmond, the pressure on universities to seek 'soft money' for short-term research contracts is a severe strain on the existing state-funded research base, as well as favouring selected areas of research to the exclusion of less commercially attractive projects.

Simon Hadlington

Chernobyl takes macaroni off Japan's menu

Tokyo

A JAPANESE consumer cooperative has stopped supplying imported Italian pasta to its customers in the wake of Chernobyl. The case illustrates the sensitivity of some Japanese to radioactivity.

The 147,000-member Seikatsu Kurabu ("Life Club") has set what must be one of the world's strictest standards for the maximum combined levels of caesium-134 and caesium-137 in food: 37 Bq kg⁻¹, more than an order of magnitude smaller than the European Commission's limit (600 Bq kg⁻¹), which some consider too severe.

The club set the level at one tenth of that adopted by Japan's Ministry of Health and Welfare because many of their members are women in their 20s and 30s who are pregnant or have infants and they have expressed fears about consuming contaminated food following the disclosure earlier this year that some foods imported from Europe (hazelnuts, spices, tea, cattle tripe and reindeer meat) have contained high caesium levels (several hundred to a few thousand Bq kg⁻¹) thought to be derived from Chernobyl fallout.

Italian spaghetti with 80 Bq kg⁻¹ was detected by members of the club in February and the club slapped a general ban on imported spaghetti and macaroni in March. The club's restrictions are not confined to imported products. Some batches of tea leaves from Mie Prefecture were found to have about 40 to 50 Bq kg⁻¹ of caesium. And 7.6 tons, or 15 per cent of the 1986 harvest, was burned at a loss of ¥ 20 million (\$140,000).

The club's members, however, would have to be real pasta gluttons or tea addicts to exceed the natural levels of radiation to which human beings are subjected (about 200 rem per year) by consuming these products. For example, at 60 Bq kg⁻¹, one would have to eat a few tons of spaghetti in a year — a daunting prospect.

David Swinbanks

• European Community member states are still wrangling over new radioactivity limits for foodstuffs to be imposed once the post-Chernobyl emergency limits expire at the end of October. Britain, France and Spain favour the caesium limits proposed by the Article 31 committee of Euratom of 4,000 Bq kg⁻¹ for dairy products and 5,000 for other foods. West Germany wants to see the present limits maintained: 370 for dairy products and 600 for other foods. The European Commission proposes a compromise of 1,000 for dairy products and 1,250 for other foodstuffs.

Kathy Johnston

Hepavax B: correction

AN article in *Nature* of 3 September (329, 6; 1987) stated that "Hepatitis vaccine contract goes to untested product". In fact, Hepavax B received extensive testing by both the Indonesian and the Korean regulatory authorities before being licensed. The statement was also made that "the Korean vaccine does not yet have the WHO [World Health Organization] seal of approval". It should be made clear that the World Health Organization is not a regulatory body, although it offers advice on occasion on whether a vaccine has been produced according to its guidelines. WHO has received no enquiry on this matter from the Korean or Indonesian authorities and no advice on Hepavax B has been given.

The article also stated that it was unorthodox of the International Task Force on Hepatitis B to declare all vaccines acceptable if approved by the national regulatory authority. In fact, a country will frequently accept a vaccine based upon licensing in the vaccine's country of origin without additional analysis by their own regulatory agencies. □

Slot-machine approach to science in Australia's upbeat budget

Sydney

AUSTRALIA'S treasurer has pulled an almost unbelievable rabbit out of the hat — a balanced budget — although he has had to sell A\$1,000 million in assets, such as a large slice of the prime real estate beneath the Australian embassy in Tokyo, to do it. The financial sector loved it, the stock market rose, interest rates fell, and the Australian dollar strengthened. For science, the news is not so good. The treatment it received prompted the Minister of Science and Small Business, Mr Barry Jones, to criticize his own government for the low priority it gave science and for approaching research funding with a "slot-machine mentality".

"After 15 years, there is no longer a Department of Science", according to Jones, who feels that science has fallen off Australia's political agenda completely. The Department of Science, which he headed until the election last July, has been relegated to the status of a junior ministry within the Department of Industry, Trade and Commerce (DITAC), with some responsibilities being moved to other departments.

Jones holds scientists largely responsible for this situation, often criticizing them for their failure to take their cause to the public. "Unfortunately, science is both politically invisible and tongue-tied", he says. He attacks the attitude of government bureaucrats in financial and coordinating departments, saying that they are hostile towards research without the immediate prospect of economic return. He says "The very concept of 'curiosity-led' research drives many into a frenzy: it is seen as little more than a hobby, like stamp-collecting or knitting, at public expense."

Hardest hit is Australia's large research agency, the Commonwealth Scientific and Industrial Research Organization (CSIRO), one body that has stayed with Jones with his move to DITAC. CSIRO's allocation has been cut from A\$364 million last year to A\$354 million this year, a cut of about 10 per cent, allowing for inflation. CSIRO is expected to make up the shortfall through sales of assets and by attracting more money from industry.

One innovation to emerge from the budget is the creation of the Australian Research Council (ARC), with a budget allocation of A\$67 million. The Australian Science and Technology Council (ASTEC) recommended the formation of ARC to coordinate diverse science-funding schemes. ASTEC suggested that A\$66 million be provided in 1987–88 to start ARC, but also said that the money should not be taken from universities' general

funds, as the level of funding is already perilously low, and that ARC should be part of the Department of Science.

The A\$67 million that the ARC has received is a sham because the government has included in it the Commonwealth postgraduate awards scheme, whose sizeable budget was not included in ASTEC's calculations, as well as taking A\$5 million from the general allocation to universities. The inclusion of the ARC in the newly created portfolio of Education, Employment and Training worries many academics because, although paying lip-service to the importance of fundamental research, the man responsible for it, ex-minister for Resources and Energy Mr John Dawkins, like senator John Button, the man in charge of DITAC, does not share Jones's passion for science. Dawkins's actual commitment to pure research will become clear when its representation on the council, whose member-

ship will be announced soon, is known.

The bright spot in this year's budget is in Button's territory, the funding of research in industry, where money for the grants for industrial development (GIRD) scheme has been increased from A\$10.8 million to A\$25.6 million in 1987–88. GIRD is for research in small, innovative, 'start-up' companies with a turnover too small to take advantage of the 150 per cent research and development tax concession.

The Department of Education, Employment and Training has been given a mandate to create 5,800 places in universities and colleges of advanced education for young school-leavers, though it is not clear where the money to do so will come from. They have, however, been given an extra A\$31 million to create 13,000 short-term traineeships aimed at young, unemployed people.

The overall position is summed up by the Australian Academy of Science, who found that although total Commonwealth Government outlays had increased by 4.3 per cent, all outlays in the budget classified as scientific research have increased by just 0.8 per cent.

Charles Morgan

Prize season begins with awards for many

THIS year's winners of the Albert Lasker awards are three molecular geneticists and a psychiatrist, while a physical anthropologist and a psychologist are among the three Balzan prize winners.

The Lasker medical research prize is for work on the genetic control of antibody diversity. It is shared by Leroy Hood of the California Institute of Technology, Philip Leder of Harvard Medical School and Susumu Tonegawa of the Massachusetts Institute of Technology. Tonegawa, a graduate of Kyoto University, carried out much of the relevant research while at the Basel Institute of Immunology, and many of Leder's contributions derive from his time at the National Institutes of Health in Bethesda.

Mogens Schou, research director of the psychopharmacology research unit of the Aarhus University Institute of Psychiatry at Risskov, Denmark, receives the Lasker clinical research prize for pioneering the use of lithium to treat manic-depressive illness.

Although the Lasker prizes are widely

respected and frequently a good predictor of Nobel prizes, they are cash-poor; Leder, Hood and Tonegawa share \$15,000. By contrast, the Balzan prize winners each receive 250,000 Swiss francs, about \$165,000. This year's winners of the prizes, which go to the arts as well as to the sciences, include Philip Tobias of the University of Witwatersrand Medical School, for his studies of hominid fossils and evolution, and Jerome Bruner, of the New School of Social Research in New York for his contributions in the field of human psychology.

Meanwhile, brothers Eugene P. Odum, of the University of Georgia, and Howard T. Odum, of the University of Florida, pick up \$250,000 at the award ceremony this week for the 1987 Crafoord prize. Awarded by the Royal Swedish Academy of Sciences within areas of science not covered by the Nobel prizes, this year it was the turn of biosciences. The Odums receive their award in Stockholm for their pioneering contributions to ecosystem ecology. □



Lasker winners (left to right) Hood, Leder, Tonegawa and Schou. Right, Balzan winner Tobias with Taung skull.

US Congress adds its voice to the genome-sequencing chorus

Washington

If Senator Pete Domenici (Republican, New Mexico) has his way, US law will require that the human genome be mapped and sequenced as rapidly as possible. But it became clear last week during hearings on a bill introduced by Domenici that the federal agencies most likely to be involved in the project, although pleased by the senator's interest, have grave reservations about his legislation.

Domenici's bill is intended to improve relations between universities and private industry on the one hand and US national laboratories on the other to speed development of technology "in energy areas of significant economic potential". In addition to the genome project, the bill would create superconductivity research centres at national laboratories, a semiconductor manufacturing initiative and a group of entrepreneurial institutes.

Acting director of the office of energy research James Decker said his agency opposes the bill as written, arguing that the superconductivity centres would be allowed to spend money without Energy Department approval. Presidential science advisor William Graham added that the White House considers the legislation of superconductivity unnecessary, because the president has already issued a directive supporting such research.

A primary goal of Domenici's bill is to improve US economic competitiveness. Using this argument, Domenici nearly succeeded in attaching his bill to a trade bill that was eventually passed by the senate. Representative Manuel Lujan (Republican, New Mexico), recently sponsored a bill in the House of Representatives identical to Domenici's bill.

The bill has sparked concern at the National Institutes of Health (NIH). As the debate over how and whether the human genome should be mapped and sequenced has proceeded over the past 18 months, there have been jealousies among government agencies about who should lead the project. The Energy Department first discussed the mapping and sequencing project in February 1986 but soon NIH, the National Science Foundation and private research organizations entered the debate.

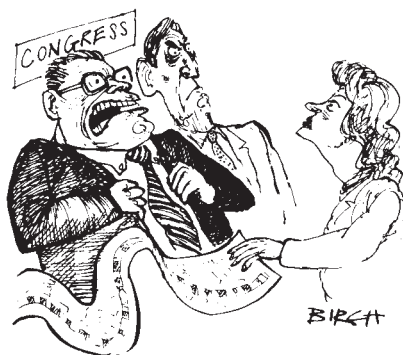
The Energy Department has a specific line item in its budget for its genome efforts. Energy Secretary James Herrington even issued a press release last week announcing that specific centres for the project had been created at the department's Los Alamos and Lawrence Livermore National Laboratories. But NIH Director James Wyngaarden has let it be known that NIH considers it presumptuous for the Energy Department to lay

claim to lead agency status. Energy will spend \$12 million on mapping and sequencing efforts this year, whereas Wyngaarden calculates that NIH spends \$300 million annually on related research.

Last week Domenici sought to reassure Wyngaarden that he has no intention of shutting NIH out of the project. He even offered to change the title of the bill to the NIH Cooperative Research Initiative if it would make Wyngaarden better disposed towards it.

Domenici's questions to the panel of scientists and industry representatives

But there MUST be a gene for Presidential Material...



testifying at the hearing centred on what specific benefits sequencing the human genome would have for US competitiveness in high technology. The industry spokesmen said they were primarily interested in the project from an engineering standpoint, and would seek first refusal to any patentable processes of inventions to come out of the effort. But Patricia Postma, a technology-transfer specialist, said new technologies emerging from national laboratories are likely to be way ahead of their potential markets, forming a barrier to commercialization.

Last week's hearing became unusually spirited, as Domenici seemed to be playing the role of prosecuting attorney, cross-examining Wyngaarden and Decker. Domenici tried to make them admit that the genome project would be more certain to proceed if there was legislation making it a national policy to do so. But Wyngaarden retorted that plenty of biomedical research is accomplished without the benefit of a specific national policy.

Wyngaarden and Decker seem to think the Domenici bill unnecessary, and that coordination of federal activities can come from a subcommittee of the White House Domestic Policy Council that Wyngaarden chairs. **Joseph Palca & Carol Ezzell**

Theory never to be practice?

Ithaca, New York

CORNELL University was naturally pleased to receive a pledge of \$10 million from the state of New York to build a new home for the Theory Center — Cornell's national centre for advanced scientific computing. But plans have hit a snag. The proposed building cuts into the tree line above one of Ithaca's famous gorges. Opponents have launched a campaign to block construction at the proposed site, throwing the university's plans to modernize its engineering campus into turmoil.

The Theory Center is one of the five national supercomputer facilities supported by the National Science Foundation (NSF). But as university officials hasten to point out, it is the only one without its own building. Theory Center staff are at present in temporary quarters in the chemical engineering building.

Crowded and antiquated facilities on the engineering campus have been particularly taxed by Cornell's success in attracting research funding. Cornell vice-president John Burness says the university leads the country in research awards from NSF, and research spending by the engineering faculty has increased nearly eight-fold in the past decade.

But the engineering 'quad' is virtually out of space. The university thought it had found a solution by building on a parking lot behind the quad. But the structure, designed by Robert Siegel of Gwathmey-Siegel of New York, did not end at the parking-lot boundary — it stretched into the gorge cut by Cascadilla Creek. Burness says the university "goofed" by not realizing this from the start.

The university ordered modifications, bringing the building closer to the parking lot. But even in its modified form, a blue ribbon marking the site of a retaining wall on one side of the building cuts through a stand of trees that marks the edge of the gorge. This is despite a promise made in 1972 by the university regents not to build into the gorges, and complaints were aired at a public hearing held on 17 August.

Burness rejects criticism that the university has not planned the building carefully. He says the university's options are limited, especially if the Theory Center is to be kept near the engineering campus. Keith Kennedy, chairman of the advisory board for Cornell Plantations which oversees the university's undeveloped property, believes the university has done the best job possible in locating the new building, but he expects the rest of his board will vote to reject the site chosen. The Ithaca city planning board has already considered and unanimously rejected it. **Joseph Palca**



Cornell's supercomputer centre, the Theory Center, will remain theoretical and in model form (left) unless the New York State Urban Development Corporation and Ithaca residents can be won over. The William M. Keck Science Building at Stanford (right) may set a trend in laboratory building. The narrow-windowed 'extra storeys' hold the key. See below.

Stanford seeking backers for reconstructing red-tile excellence

Palo Alto

STANFORD University is bent on breaking all records for the private financing of scientific research facilities. A drive is under way to raise \$250 million for the complete reconstruction of the 'near-west' science and engineering region of the university campus. And that is just one part of a campaign aimed at raising \$1,000 million by the one hundredth anniversary of the university's founding in 1991.

Like many US universities, Stanford is witnessing the decay of science facilities built during the 1960s 'post-Sputnik' boom when success in science became a cold war imperative. Stanford's president, Donald Kennedy, who as a neurobiologist and a former head of the Food and Drug Administration can fairly claim to know Washington science politics as well as laboratory science practice, is a noted critic of the administration's failure to "spend anything on renewing university science facilities".

While Kennedy is likely to remain a lobbyist for increased federal spending on research facilities, his solution at Stanford is to try to double the rate of private fundraising through into the 1990s when the billion-dollar goal can be reached.

That solution may not be open to many other private universities. Stanford is one of the few with the connections that make it possible to raise enormous sums of money. Stanford is also under pressure to maintain high standards: there are 13 Nobel prize-winners on campus and a lot of competition for top scientists both from the nearby University of California and from big east coast universities.

Two buildings that will stand on the near-west campus have already been completed "on time and without any government help" as Kennedy puts it. One is the Center for Integrated Systems, where Sili-

con Valley companies have provided the means to design the next generation of very-large-scale integrated circuits. The other, the Keck Building, is to be the cornerstone of the new campus. Specially designed for flexibility, it will provide a home to researchers displaced from laboratories that are being rebuilt.

Even though the new science campus will eventually be larger than the whole of the Massachusetts Institute of Technology, Stanford staff are insisting that it must retain the "spiritual qualities" of the old Stanford — despite the fact that nine older laboratories are to be demolished. Tall concrete and glass buildings are not favoured in the grand plan. Instead, low

buildings are to be set around courtyards, with taller, three- or four-storey buildings further back. The courtyards, including one with a fountain and pergola, will be connected by tree-lined alleys linked to the rest of the campus, ending science's isolation behind a long wall of laboratories. Even the traditional red tile that is the hallmark of Stanford's oldest buildings is likely to be used.

The sequence in which buildings will go up will depend on finding a solution to a complicated game of musical chairs, with the Keck building providing the empty chair. New buildings will not reflect departmental boundaries but a series of disciplinary clusters: physical and engineering sciences, information sciences and, closest to the medical faculty, biochemical sciences. The aim, Kennedy says, is to anticipate future growth areas "by providing flexibility". **Alun Anderson**

Architecture for molecular medicine

Palo Alto

"MANY desire entry, but few will be chosen." If local gossip is to be believed, the quotation may be apt for the new Center for Molecular and Genetic Medicine under construction alongside the Stanford University Medical Center.

Under the direction of Professor Paul Berg, almost all of the \$90 million needed to build the institute and to endow it for its first five years has already been raised. Part of it will be occupied by a Howard Hughes Medical Institute Unit with a dozen staff handsomely provided for. Elsewhere, there will be three research departments — biochemistry, developmental biology, and molecular and cell physiology — with nine or so staff each. Adding on assistants, postdocs, technicians and administrators will give the building a population of nearly 700.

Some staff will come from the medical centre but new people from outside Stanford will also be welcome — fresh

talent is needed before the present generation of Nobel prizewinners wears out.

The centre's title looks to the future opportunities to build bridges between molecular biology and medicine. High-speed elevators and wide, gracious stairwells are being designed to ensure that molecular and medical factions have no difficulty in meeting to exchange ideas.

And a glance at the building reveals that, just like the Keck building, there is something odd about the spacing of the floors. An unusual row of circular windows below the main windows is the give-away. In fact, it has borrowed from the idea of the Keck Building's Scottish architect, Jim Borthwick, and put in "interstitial floors", each high enough for a man to stand in and carrying the pipes and ducts. It makes the building taller but that extra construction cost should be recovered in lower installation costs and the a greater ease with which laboratories can be put to new purposes.

Alun Anderson

Boycott of South Africa

SIR—We are white South African scientists working quite openly for the removal of the present government, but we do not welcome the scientific boycott, as J.G. Wilson (*Nature* 328, 288; 1987) presumes we should. Wilson also presumably includes us amongst the whites “who choose to enjoy the advantages of living comfortably in South Africa, at the expense of the black population”. We can assure him that we, and many of our colleagues, would be more comfortable, and more fulfilled as scientists, if we were living at the expense of the population in Europe, North America or Australia. It is far from comfortable being a scientist in South Africa today, being perceived as largely irrelevant by most of those engaged more directly in the current political turmoil, and pariahs by many fellow scientists abroad. Those of us who could move would be elsewhere, if we were motivated by comfort or short-term personal gain.

We agree with Wilson that the scientific boycott would encourage native white South African scientists to leave the country, not “for the duration”, as he glibly remarks, but forever. The best scientists would be most likely to find employment elsewhere. Such emigration, which is occurring already, and other effects of the boycott, would damage science and technology immeasurably in South Africa. We suspect that the least damage would be inflicted in the military area, both because the country is probably more self-sufficient in military science and technology than in any branch other than mining, and because military hardware is always available, at a price. What will suffer most is the science and technology that seems least critical in the short-term: medical science, agriculture, ecology and conservation, and especially science education. Despite Wilson’s contention to the contrary, science education and scientific careers in South Africa are not the preserve of whites, and to claim that they are is derogatory to black South African scientists. However, science education still depends largely on white lecturing and teaching staff. If they were to be depleted, many South African blacks would continue to be deprived of the opportunities in science and technology from which they have been excluded in the past by the structures and philosophy of apartheid. Wilson seems to favour such an outcome, but we doubt whether he would receive the support of the majority of black students in our classes.

We hope that readers of *Nature* will agree that no country is likely to attain a quality of life acceptable to the majority of its people without a commitment to and competence in science and technology,

whatever the country’s political persuasion. Wilson’s clairvoyance does not appear to extend to plans for providing such competence in a future South Africa, from which the scientists he has encouraged to leave have departed. Examination of other developing countries in Africa shows how hard it is for such countries to bootstrap themselves into first-world science, and also that they cannot rely on first-world immigrant scientists to help them do so. It seems that many qualified scientists find it more comfortable to be on the dole in the United Kingdom and elsewhere than to be contributing to science and science education in developing countries. We believe that the future South Africa will have scientific and technological competence only if South African scientists of all colours stay here and work for it; that is why many good scientists have chosen to stay, in spite of the personal cost.

Wilson appears to like his dilemmas posed in simplistic terms. Our simplistic version of the dilemma facing those having to take sides on a scientific boycott of South Africa is this: do you wish all South Africans to have access to the first-world quality of life now enjoyed by only a minority, or do you wish none to do so?

DUNCAN MITCHELL

HELEN LABURN

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SIR—J.G. Wilson outlined “. . . some arguments for the scientific boycott of South Africa” (*Nature* 328, 288; 1987). Apart from containing factual inaccuracies, Wilson’s letter reflects a simplistic view.

The motivation for being nasty to white South African scientists appears to be the notion that this will somehow help blacks in South Africa. Will it? White South African scientists do not run the country. It is the South African government that does so. The former show a great interest in matters such as scientific boycotts whereas the latter, in the short term at any rate, has little reason to take any notice whatsoever of any scientific boycott. The government is trying to sort out issues it would consider to be more important than the relegation to the dog-box of the comparatively small community of South African scientists. Consequently, a scientific boycott is unlikely to hasten any “. . . overthrow of the present system”.

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

Should white South African scientists find that they are unable to publish in journals of their choice, many will simply leave the country (and many have already done so). Few will return. The future black scientists and academics of South Africa will then inevitably be third-rate, and will be employed at what will have become fourth-rate institutions.

M.B. MARKUS

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Creationism lives

SIR—The National Center for Science Education is pleased with the Supreme Court creationism decision (see *Nature* 327, 645; 1987). Now teachers in the state of Louisiana will not be forced to present biblical literalism as science. Reports of the death of “scientific creationism”, however, are premature. The Supreme Court decision says only that the Louisiana law violates the constitutional separation of church and state; it does not say that no-one can teach scientific creationism—and unfortunately many individual teachers do. Some school districts even require ‘equal time’ for creation and evolution. A number of organizations exist to encourage teachers to introduce ‘scientific’ creationism, and they are not deterred by the failure of the Louisiana legislation. The strength of the creationist movement has always been at the grassroots level, and this is where it will continue to thrive. Those supporting science education also need to focus their efforts at the grassroots level, by becoming involved in textbook selection, supporting improved teacher training and being aware of what actually is taught in the classroom.

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Not so bad

SIR—No doubt NASA (National Aeronautics and Space Administration)’s latest mishap at the Wallops Island Flight Facility may leave us wondering if ‘mother Nature’ has decided to take part in the plot against the space agency also (see *Nature* 327, 543; 1987).

But look at it this way: three launches at once, two of them successfully taking their prescribed course and the third one ‘launched’ even without a launching pad, may be considered quite a strike, lightning notwithstanding.

JORGE GOLOWASCH

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Prospects for fifth force fade

Conflicting observations of the gravitational-like interactions which have engendered talk about the fifth force might have been reconciled if it were not for another set of data.

WHATEVER has happened to the fifth force? Eighteen months have passed since the existence of a non-newtonian interaction between massive objects was postulated by Fischbach, Sudarsky, Szafer, Talmadge and Aronson on the basis of their reanalysis of the measurements by Eötvös, in the 1920s, of the apparent 'gravitational' interaction between objects of various composition.

That the old data are shot through with anomalies is not surprising. What Fischbach *et al.* say is that the anomalies could be removed if there is an extra interaction between material objects whose strength is determined not by the mass of an object but by its content of baryons (neutrons and/or protons) which, because of nuclear packing fractions and non-zero electronic masses, is not related to the total mass of objects in the same way for all materials.

At the outset, the advent of the fifth force was both welcome and disconcerting. On the face of things, it has the advantage of being able to account for the way in which geophysical measurements of the gravitational constant, where one of the sources of gravitational attraction is an object such as the Earth, tend to be consistently higher than those derived from laboratory measurements (see *Nature* **319**, 173; 1986). Among theorists, the development was also welcome on general grounds of completeness: baryon number, like net electrical charge, is conserved in the standard particle theories, but appears not to play a part in the determination of an interaction between objects. The bad news was merely that a non-newtonian interaction (with a short range somewhere between a few metres and 10 km) would have been an unwelcome, if intriguing, complication of an orderly world.

Either way, it has been clear that the issue will be settled only by experiment, which explains why so much ingenuity has been lavished on novel measurements in the past 18 months. Simply repeating the Eötvös measurements has naturally not been appealing. Given that the supposed force is very small compared with authentic gravitation, the best hope of detecting it rests on measurements of apparently gravitational attractions in circumstances where there is a great bulk of matter generating fifth force in some recognizable direction.

One of the most ingenious schemes (due to Professor S.K. Runcorn of the

University of Newcastle upon Tyne) would entail the measurement of gravitational acceleration near the energy storage plant at Dinorwic in North Wales; gravimeters near the site should behave differently at different distances from the reservoir filled with water at off-peak times in the demand for electricity in Britain. Others have been busy off the faces of cliffs or beneath hillsides that spring out of an otherwise uniform topography. Unfortunately for the fifth force, the results so far have been mixed.

Indeed, the two experiments so far described have given flatly contradictory results. Thus P. Thieberger, working on an observation platform on the cliff-face of the Palisades, found a horizontal force between the cliff-face and a copper sphere floating in a tank of water (*Phys. Rev. Lett.* **58**, 1066; 1987). On the other hand, a group from the University of Washington (C.W. Stubbs *et al.* *Phys. Rev. Lett.* **58**, 1070; 1987) reported measurements with a copper and beryllium torsion balance installed on a hillside on the Seattle campus which flatly contradicted Thieberger's result or which, more accurately, fixed an upper limit for the magnitude of the supposed fifth force at two orders of magnitude less than that inferred from the reanalysis of the Eötvös measurements.

What, in such circumstances, should experimentalists do? The standard procedure is to pick over the other set of data, looking for sources of systematic error that might account for the discrepancy, a process that can be quite acrimonious. On this occasion, the Seattle group, almost as if it regretted having helped to make an interesting problem go away, has followed an ingenious tack by asking "What if we are both right?" (see Adelberger, E.G. *et al.* *Phys. Rev. Lett.* **59**, 849; 1987).

How could that be? The suggestion is that the 'charge' responsible for the fifth force is not simply the baryon number of an interacting object but some combination of baryon number, B , and lepton number, L , conveniently (and without loss of generality) written as $q = B\cos\theta + L\sin\theta$. What this implies is that the magnitude of a fifth-force attraction will be determined by the ratio of baryons to leptons in the test material. Given two accurate measurements, at Seattle and New York, it is plainly possible to find some value of the angle θ that will reconcile the null result at Seattle with the

positive result off the Palisades cliffside. For what it is worth, θ comes out at -11° .

So are the two apparently conflicting measurements merely manifestations of the same underlying phenomenon? The snag is that, while it may be possible to fit a sine curve to two arbitrary points, the same fit can also be used to define a more sensitive test of how good the fit may be. Adelberger *et al.* now simply figure out from their sine curve that, if the copper and copper/beryllium results can be reconciled at $\theta = -11^\circ$, replacing the copper component of their torsion balance by aluminium should give a positive result under the same condition, although not as large as that of Thieberger.

The result is again a disappointment for believers in the fifth force. The torsion balance constructed from beryllium and aluminium spheres (two of each, arranged asymmetrically to form a fifth-force charge dipole) gives a null result, or one smaller by two orders of magnitude than required to account for Thieberger's result.

Where the argument will go from here is a matter of conjecture, although the stimulus engendered by excitement about the fifth force for accurate measurements of gravitational acceleration (g) under widely different circumstances may indeed soon lead to a better understanding of why the gravitational constant (G), which fixes the magnitude of the newtonian gravitational force, is still among the least well defined of the natural constants.

If, by good luck, a much more accurate value of G should emerge, we should all be the gainers. On the theoretical side, the case for believing that baryon and lepton numbers should have roles somehow analogous to electric charge, while only a formality in most arguments so far, should not be too lightly dismissed. Why not?

The obvious difficulty is that, if these numbers separately or jointly are to function as charges acting as sources for an interaction between material objects, the force will have to be mediated by particles of some kind. The snag here is that the particle called the axion, the existence of which is predicated by the strict application of parity and time reversal in weak interactions, and which has been canvassed both as a mediator of the non-newtonian gravitational force and as the constituent of 'dark' intragalactic matter, has been having a bad time recently in attempts to demonstrate its experimental reality.

John Maddox

Materials science

Novel model for cavity lattices

Robert W. Cahn

MICROSCOPIC cavities, either empty (voids) or gas-filled (bubbles), created in crystalline solids by irradiation or ion implantation, are apt to form lattices that mimic the host lattice but are coarser by a factor of 70–120. These entities are termed void or bubble lattices; cavity lattice covers both categories. Void lattices were originally discovered in molybdenum by Evans¹ at the Atomic Energy Research Establishment at Harwell, United Kingdom, where much of the subsequent work has been done. Cavity lattices have been observed in metals with various structures, alumina and alkaline earth halides. Johnson *et al.*², also working at Harwell, on page 316 of this issue, propose a radically new mechanism, an inter-cavity repulsion caused by 'dislocation punching'. This joins several other mechanisms that have been proposed (reviewed in refs 3,4) to account for the formation of cavity lattices.

Cavity lattices in cubic metals are always in 'parallel orientation' and fully developed in three dimensions; in hexagonal-close-packed (hcp) metals, however, voids aggregate in sheets parallel to the basal plane, without internal ordering in the sheets. Most current theoretical models address the formation of parallel-oriented cavity lattices. It was recognized soon after Evans's original discovery that an elastic interaction between voids or bubbles arises from the anisotropy of the elastic constants; there are both attractive and repulsive components so that an optimum separation between cavities results. But elastically isotropic metals like tungsten also form voids, a difficulty that is overcome if the spherical voids are allowed to distort and develop flat faces. Although the stability of a lattice, once formed, can certainly be explained in terms of this rigorously developed static model, it is still uncertain whether the energy minimum at the preferred cavity separation is deep enough to permit lattice formation in the first place on the basis of this model (J.H. Evans, personal communication).

Dynamic theories

More recently, dynamic theories have been developed on the basis of anisotropic diffusion of self-interstitial atoms (SIAs); in the original form, constrained one-dimensional diffusion or dynamic replacement sequences were assumed. This kind of mechanism can be effective because unaligned voids will receive a greater flux of SIAs than those which are already aligned, because the latter will be locally

shadowed by cavities already in the lattice. Cavities migrate in a crystalline solid by a mechanism involving surface self-diffusion along the cavity wall — the more rapidly, the smaller the cavity — and the flux of SIAs accelerates such migration. Local shadowing acts as a feedback control mechanism tending to perfect a lattice. The most recent variant of this approach, originally proposed in 1972, is by Woo and Frank⁵. This kind of model has also been embraced by those seeking to interpret cavity lattice formation in non-metals⁴.

An alternative form of SIA model, recently advanced by Evans⁶, involves two-dimensional diffusion of SIAs confined within a crystal lattice plane. Computer simulation has shown that this kind of motion, for which there is sound theoretical basis, can by degrees convert a random cavity array into an ordered one. This is the only model up to now that can explain the planar ordering of cavities in hcp metals. Unlike the elastic anisotropy approach, however, this model is less clear about the magnitude of the interaction holding the lattice in place once it is formed. The older model interprets stability, the newer ones concentrate on initial formation.

The latest Harwell model is intended to interpret a novel set of observations, also reported in the paper of Johnson *et al.* in this issue². The authors injected helium into copper (a venerable technique, first used in 1960 by Barnes and Mazey — the latter is one of the authors of the new paper — in the demonstration of bubble mobility in a solid metal). Helium bubbles about 2 nm in diameter are created at a concentration of about 10^{25} m^{-3} — an unusually high concentration — and form a bubble lattice, accurate enough to give satellite maxima in electron diffraction. What is novel about this lattice is that parts of it are *not* in parallel orientation: two types of domain are present sharing a common [111] plane with the parallel-oriented domains, but are mutually rotated about the perpendicular <111> direction. This is the first firm observation of cavity lattice domains in non-parallel orientation.

The authors go on to point out that small noble-gas bubbles formed by ion implantation are always grossly overpressurized, in the sense that the surface tension alone does not suffice to contain the gas pressure. The crystal surrounding each bubble is consequently under great stress. Such bubbles can grow by absorbing vacancies created by radiation damage

caused by the implanting beam, but when these are exhausted, the only further way a bubble can grow (and thus reduce its overpressure) is either by merging with another bubble — not feasible in a lattice with repulsion between bubbles that approach each other too closely — or by plastically deforming the stressed crystal matrix surrounding the bubble.

One mechanism of plastic deformation that has been well documented^{7,8} is dislocation punching: a succession of mobile dislocation rings, of the same diameter as the bubble, is dispatched from the bubble along a 'glide cylinder', the axis of which coincides with the Burgers vector, <011>, away from the surface of the bubble, providing more room for it.

Mutual repulsion

Thus, neighbouring helium bubbles disposed so that a <011> vector joins them, experience a mutual repulsion from the dislocation rings which each bubble emits towards the other, as has indeed been previously recognized⁹. (Dislocations of the same sign necessarily repel each other and their sources.) This mutual repulsion may suffice to overcome the elastic attraction which holds the bubble lattice together, and the authors demonstrate² that this process will convert the parallel orientation into one or other of the two observed alternative orientations, in both of which the bubble separations parallel to <011> are increased by a factor of at least $\sqrt{3}$. The experiments reported in this issue involve particularly high helium concentrations and close bubble spacings (about 6 nm) so that bubble repulsions should be unusually strong.

Studies of cavity lattices such as this have a bearing on the ordering, on lattices, of other structural entities. Khachatryan and Airapetyan¹⁰ have demonstrated that elastic interactions can account for the formation of precipitate lattices. In principle, precipitates could also stress the surrounding matrix crystal lattice if their unconstrained atomic volumes exceed that of the matrix lattice, and conceivably dislocation punching could also be involved in the formation of this kind of macrolattice. □

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Astronomy

New millisecond pulsar in an unusual environment

David J. Helfand

THE discovery of millisecond pulsars, neutron stars with rotation frequencies of 100–600 Hz, is still sufficiently rare for each new detection to be a cause for a minor celebration among pulsar aficionados. The detection of the fourth such object a few weeks ago, however, was of interest to a wider circle of astrophysicists, because of its very special location. PSR1821–24, reported by A.G. Lyne *et al.* (*Nature* **328**, 399–401; 1987) to be spinning at 327 Hz, is situated in the core of a relatively dense star cluster, M28. As such it provides new information on the origin and evolution of these remarkable objects, as well as offering the first chance for an *in situ* probe of globular cluster dynamics. Already theorists are proposing explanations for the peculiar properties of the pulsar, as exemplified by three papers elsewhere in this issue.

The first pulsing radio source was recorded 20 years ago as the famous “little bit of scruff” on Jocelyn Bell Burnell’s chart records, and since then, nearly 450 objects with periods between 0.033 and 4.5 s have been located. Accepted soon after their discovery as the first unambiguous manifestation of neutron stars, these pulsars have revealed a wealth of information on subjects as diverse as the structure of nuclear matter and the structure of the Galaxy’s magnetic field, and have served as important constraints on theoretical constructs ranging from stellar evolution to general relativity. The pulsed character of their emission arises as a beam of radiation, defined by the star’s intense magnetic field, and sweeps past our line of sight once each rotation period. The energy to supply this beacon is derived from a slowing of the star’s rotation period.

Wide interest in pulsar astronomy was rekindled just five years ago with the announcement by D.C. Backer *et al.* (*Nature* **300**, 615–618; 1982) that they had discovered a pulsar with the astonishing rotation frequency of 642 Hz. Subsequent observations showed that this source had a spin-down rate of less than 0.3 ns per century, and monitoring over the intervening years has established that, as a clock, this object rivals in accuracy the banks of atomic oscillators used to establish the World’s time standard. This remarkable stability has, among other things, been used to set a stringent upper limit on the energy density of gravitational waves left over from the moment of the creation of the Universe.

Some time after the discovery of this original millisecond pulsar, two more objects with ultra-short periods were found, both members of binary systems. This fact, coupled with the anomalously low magnetic fields of all three objects and their extremely rapid rotation rates led several authors to suggest that such pulsars, in fact, were born with nearly normal properties, spun down, and died like other pulsars, but were then ‘reborn’ when accretion of matter from a binary companion spun them up again.

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The Milky Way region of Sagittarius, showing the star λ Sagittarii (left), and the globular cluster M28 (right) in which the new millisecond pulsar PSR182124 was discovered recently.

Stimulated by this suggestion, T.T. Hamilton, R.H. Becker and I (*Astr. J.* **90**, 606–608; 1985) undertook a search for candidate millisecond objects in the dense cores of globular star clusters where the appropriate type of progenitor binary systems are 100 times more numerous (per star) than they are in the rest of the Galaxy.

Only one of the twelve clusters searched turned out to contain a point-like radio source, hardly more than were expected based on a chance alignment with extragalactic objects. The spectrum of the radio emission of this one source was unusually steep, however, and subsequent observations at lower radio frequencies by W.J. Erickson and M.H. Mahony (*Astrophys. J.* **299**, L29–L31; 1985) and by a collaboration of these two sets of authors (Erickson, W.J. *et al.* *Astrophys. J.* **314**, L45–L47; 1987), found the emission to be highly polarized with a distinctly pulsar-like spectrum. Finally, more than 4 years after the source in M28 was first detected, a herculean effort, involving many hours of observation and 16-million-point Fourier transforms, led Lyne *et al.* to announce the discovery of a pulsar with a periodicity of 3.05 ms, adding the fourth member to the millisecond-pulsar family.

Even before the M28 source was confirmed as a pulsar, R.D. Blandford, R.W. Romani and J. Applegate (*Mon. Not. R. astr. Soc.* **225**, 51–53P; 1987) pointed out the potential advantage of having a precise clock moving through the gravitational field of a dense star cluster. The cluster core has a density of several thousand stars per cubic light-year and, as the pulsar moves through this stippled potential it will experience acceleration from passing stars that will double in 1,000 years. The change in acceleration, then, will be measurable on a timescale of about 3 years as an anomalously large period second derivative. Thus, a long-term timing programme for such a pulsar could provide a direct, *in situ* measurement of the cluster’s stellar density. An ancillary product of such timing data will be a determination of the cluster’s motion in the plane of the sky, offering the first chance to determine a three-dimensional velocity for these old stellar systems.

Although it was the binary model of millisecond pulsar formation that led to the discovery of the M28 pulsar, this source has no binary companion. In this issue, three sets of authors explore the consequences of this fact for the pulsar’s evolution and assess the likelihood that more such objects will be found in globular cluster cores.

F. Verbunt *et al.* on page 312 of this issue explore possible models of the evolution. They set an upper limit to the pulsar’s magnetic-field strength by adopting the standard accretion spin-up model and requiring the object to be younger than the Universe; for a field strength equal to that of the other three millisecond objects, they note that the accretion era must have terminated only 750 million years ago, less than 5 per cent of the age of the cluster (and probably of the neutron star as well). They enumerate the types of collisions that could lead to the formation and subsequent disruption of a binary system capable of spinning up a primordial cluster neutron star. A head-on collision with a normal star should lead to disruption of that star and the formation of a massive disk around the pulsar, whereas glancing collisions can result in close binary systems. The two schemes lead to different predictions for the number of millisecond objects visible in clusters today, and searches in progress may well rule out the massive-disk picture if only a few new objects are found.

F.C. Michel presents an iconoclast’s view of the M28 pulsar’s origin on page 310 of this issue. He argues that the magnetic-field evolution required by the standard picture is on shaky ground, both theoretically and observationally, and that there is no *a priori* requirement that this neutron star be very old. Instead, he proposes that the pulsar could have formed in a white-dwarf binary in which

one of the stars is pushed over its stability limit and collapses to a neutron star. Although white-dwarf binaries are one of the popular models for the supernova explosions seen in the old stellar populations, such as those which make up globular clusters, the standard picture has the exploding star disrupting completely, not collapsing to form a pulsar. One branch of Michel's scheme has the newly formed neutron star spinning its companion into an encircling disk (a prerequisite for pulsar action in Michel's favourite model) and seems a touch contrived, but the value of an alternative to the canonical picture is clear.

R.W. Romani, S.R. Kulkarni and R.D. Blandford take us back to a more conventional view on page 309 of this issue, where they explore ways to rid the pulsar of its erstwhile companion and relate the millisecond-pulsar formation rate (based on this one detection!) to the observed frequency of the X-ray-emitting sources that mark the accretion (that is, spin-up) phase. Assuming that 300 neutron stars remain bound to a cluster after their formation in supernova explosions, these authors find that approximately 20 are captured into binaries by encounters with

other cluster stars. Following various subsequent evolutionary schemes, the authors conclude that among the 100 dense clusters in the Galaxy that harbour 10 X-ray binary sources, we should expect roughly 10 millisecond pulsars to be found in wide binaries, and another three to be detectable as isolated objects.

Continuing timing observations for the M28 source will yield stringent limits on any residual disk material in orbit about it and may provide a direct measure of the accelerations caused by passing cluster members. When combined with observations of the original millisecond pulsar located more than 1 rad away across the sky, these data will provide the first legs of an anticipated reference system based on such objects which can be used to refine the solar system ephemeris and to set ever more stringent limits on the background gravity waves. Meanwhile, searches are under way in an effort to locate more such objects in clusters, to extend the unique view they provide of stellar evolution and binary formation in these ancient star systems. □

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Molecular biophysics

Going round in receptor circles

Arthur Karlin

THE nicotinic acetylcholine receptor contains a cation-conducting channel, the opening of which is regulated by the binding of acetylcholine. By electron-image analysis of two-dimensionally ordered arrays formed in receptor-rich membrane, Unwin and his colleagues have determined¹ the overall shape of this receptor and the orientation of its five membrane-spanning subunits. In their latest work, recently reported in the *Journal of Cell Biology*², they have determined the order of the subunits around the channel.

Langley first invoked a 'receptive substance' for nicotine (14 years before the discovery of acetylcholine) to explain the contraction of skeletal muscle exposed to nicotine and the block of the nicotine effect by curare. Eighty years later we have the tools, if not yet all the facts, to reach an understanding of the function of the nicotinic acetylcholine receptor (AChR) in terms of its molecular structure. To assign functional roles to subunits, subsequences and individual amino-acid residues, we have, on the one hand, affinity labelling and peptide separation and sequencing³ and on the other, gene cloning and sequencing and the functional expression of genetically altered receptors⁴.

The prerequisite for a complete molecular explanation, however, is a three-

dimensional map of the structure at atomic resolution. Several groups, encouraged by the success of Michel and co-workers with the photosynthetic reaction centre⁵, are trying to grow AChR crystals suitable for X-ray analysis. Although it is difficult to obtain well-ordered three-dimensional crystals of intrinsic membrane proteins, ordered two-dimensional arrays of several of these proteins either occur naturally, like bacteriorhodopsin, or can be induced, like AChR. After a few weeks at room temperature, about 10 per cent of native membrane vesicles isolated from electric tissue of the ray *Torpedo* form tubular sheets of ordered AChR^{1,6}.

Brisson and Unwin¹ obtained electron micrographs of these ordered arrays of AChR both in negative stain, outlining the domains outside the membrane bilayer, and in ice, outlining the entire protein. The specimens were tilted at angles of up to 65° to the electron beam, and the images combined by Fourier transformation and synthesis calculations to yield a three-dimensional structure (*a* in the figure). The entire structure fits into a cylinder, 80 Å in diameter and 140 Å long. The wider extracellular domain contains a central, water-filled channel, 30 Å wide at the extracellular end and narrowing within the bilayer. Its continuation to the

cytoplasmic end of the AChR has not been resolved, but it would be hard to explain an extensive body of electrophysiological data if the continuation did not exist. On faith, then, there is a relatively narrow channel from mid-membrane to the cytoplasmic end of the AChR. It is at least 7 Å wide, cation-specific and gated. The binding of acetylcholine at sites a considerable distance away in the extracellular domain opens this channel.

Brisson and Unwin¹ also found that the five membrane-spanning subunits of the receptor are arranged regularly around the central axis (the channel) for about half of their length, sufficient for the AChR in projection to have near pentagonal symmetry (*a* in the figure). The subunits are not well-resolved at the extremities, however, especially at the cytoplasmic end of the structure.

The subunit composition of the receptor is $\alpha_2\beta\gamma\delta$; each α contains an acetylcholine-binding site⁷. The four sequences optimally aligned are about 50 per cent identical or conservatively substituted⁸. Each has four stretches of mainly hydrophobic residues likely to form membrane-spanning α -helices⁹, a motif recently found in two other ligand-gated channels (see the recent discussion in News and Views⁹). Although the direct evidence concerning the folding of the AChR subunits in the membrane is incomplete, the similarity of these four candidate membrane-spanning subsequences from subunit to subunit is consistent with a pentagonally symmetrical arrangement of the subunits around a membrane-spanning channel^{8,10}.

The order of the subunits round the channel is relevant to the assembly and interactions of the subunits and to the structure of the channel. It was assumed that the central 30-Å pit seen in negatively stained AChR¹⁰ is the entrance to the channel and that the subunits surround the channel like barrel staves, assumptions largely substantiated by Brisson and Unwin's results. Attempts by Zingsheim¹¹, Stroud⁶ and my own group⁷ to determine the order of the subunits began about six years ago. Individual subunits were identified in electron micrographs of negatively stained AChR by various tags, the α -subunits, for example, by the increased stain exclusion caused by the binding of α -bungarotoxin¹¹ or by the binding of biotinylated cobrotoxin plus avidin⁷. The consensus was that the two α -subunits are not next to each other.

All these studies used AChR from *Torpedo* electric tissue, which occurs naturally as a disulphide-crosslinked dimer. Isolated and negatively stained, the dimer looks like two doughnuts stuck together^{7,10} with the disulphide bond between the δ -subunits. The δ -subunits were thus assumed to be at the region of closest approach of the AChR monomers

in a dimer. From the location of the α -subunits, tagged with avidin-biotin-cobrotoxin, relative to the crosslink in the dimer, we concluded that the α -subunits do not bracket the δ -subunit. From the formation of dimers crosslinked through β -subunits artificially, and from the location of the α -subunits in this dimer, we concluded that the α -subunits do not bracket the β -subunit. By elimination, we concluded⁷ that γ must be the single subunit between the α -subunits and that the order around the channel is $\alpha\gamma\alpha\beta\delta$ (c in the figure, alternative arrangement).

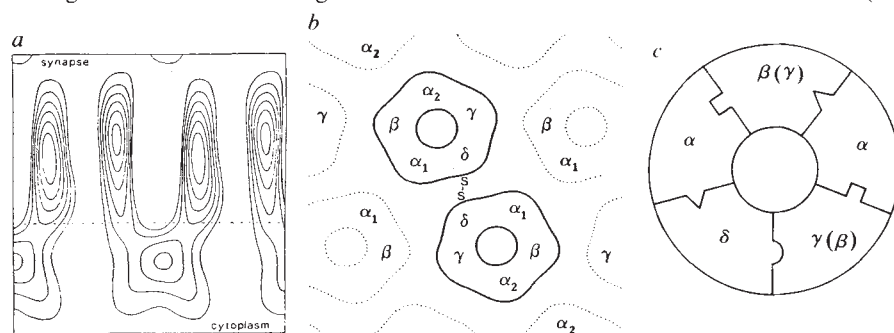
In contrast, Unwin and colleagues² have now concluded, as others had on less strong evidence^{6,11}, that the order is $\alpha\beta\gamma\delta$ (b in the figure). Unwin *et al.* tagged the α -subunits with either α -bungarotoxin or a Fab fragment of a

be misassigned if its determinant were azimuthally displaced sufficiently on either side of the membrane. Unwin believes this is unlikely, but the pentagonal alignment of the subunits has not been seen on the cytoplasmic side, and the extent of intermingling of the subunits is unknown.

A general problem with antibody tags for the AChR subunits is that their specificity is tested against denatured subunits or subsequences, but these antibodies are used at 100-fold higher concentration to tag the native membrane-bound AChR. It is difficult to test the specificity of antibodies for the native subunits because no one has succeeded in dissociating the AChR except under denaturing conditions. In addition, even monoclonal antibodies frequently show cross-reaction with different denatured subunits (for

subunit-interaction sites on its two sides and would form stable homo-oligomers. Even if this simple reasoning applies to the electric-tissue and skeletal-muscle AChR, a neuronal AChR with only two kinds of subunits has recently been described⁹. Its subunit stoichiometry is not yet known, but if a pentamer is required¹² there may be more complex subunit interactions, as in bacteriophage tail assembly¹³.

Recent work by Numa's group¹⁴, moreover, shows that functional electric-tissue AChR can form from just three of the four subunits. This group injected *Xenopus* (toad) oocytes with various combinations of *Torpedo* AChR-subunit-encoding messenger RNAs, and after a few days tested the functional expression of these incomplete AChRs. The injection of α -specific messenger RNA alone results in weak α -bungarotoxin-binding activity. The combination of α and either γ or δ shows more α -bungarotoxin-binding activity and also agonist-binding activity. It seems that γ or δ , but not β , promotes the conformation of α necessary for acetylcholine binding. Finally, the combination of α , β and γ has acetylcholine-gated channel activity at about 10 per cent of the complete AChR, and α , β and δ at about 0.5 per cent. These lower activities are only partially explained by lower surface densities of the incomplete receptors. The oligomeric structures of these incomplete receptors are not known, but it is a reasonable assumption that they are also pentamers in which γ and δ substitute for each other, albeit not very effectively. (In the minimal subunit interaction model, c in the figure, non-adjacent subunits, having one subunit-interaction site in common, are more likely to substitute for each other.) One of the implications of this work, then, is that when all four subunits are available, a unique oligomeric arrangement predominates. This and the other work I have discussed here illustrate how a molecular description of the AChR is emerging from the interplay of functional, biochemical, structural and genetic approaches. □



a, Cross-section through the AChR channel axis, perpendicular to the plane of the membrane¹; b, subunit arrangement seen from the extracellular (synaptic) side of the membrane²; c, schematic model of the specific subunit interactions (alternative subunit arrangement in parentheses).

monoclonal antibody against an extracellular determinant; the δ -subunit with wheat-germ agglutinin, binding to an extracellular oligosaccharide moiety; and the β -subunit with a Fab fragment of a monoclonal antibody against a cytoplasmic determinant. They bound these tags, one at a time, to ordered two-dimensional arrays of AChR, obtained the untilted electron images of the negatively stained arrays, and determined the Fourier difference maps resulting from the binding of each tag. They calculated the statistical significance of the Fourier differences and identified the most significant differences with the binding site for each tag, which they mapped on the reconstructed, *en face* image of the AChR. They thus identified tag binding sites on α , β and δ , and assigned the remaining position in the pentamer to γ .

This work has a clear advantage over previous studies in that the images of thousands of well-ordered AChR are averaged in each Fourier map. There are potential problems, however, in this as well as in the previous attempts to locate the individual subunits. The tags are as large as the subunits. Even α -bungarotoxin, which has a molecular mass of only 8,000, extends over 30 Å. The tag for β binds to a cytoplasmic determinant and, as Unwin and colleagues point out², would

example, Fig. 2 of ref. 2). Multiple tags for each subunit, both extracellular and cytoplasmic, could be the solution, but these are not readily available. Unwin and colleagues² tried several Fabs directed against cytoplasmic determinants, but the anti- β Fab was the only one that worked. Despite these notes of caution, Unwin and his colleagues have produced the strongest evidence so far on the subunit order.

In attempting to determine the order of the subunits, it has been assumed that there is a unique order, which implies unique subunit interactions. In a minimal model, three specific subunit interactions are required, one each for the two sides of the α -subunits and one for the interaction between the two non- α -subunits (c in the figure). If the interactions of the subunits are as simple as pictured, we can rationalize the separation of the α -subunits and the presence of three other subunits. If the α -subunits were adjacent, then their sites of subunit interaction would be complementary, and they would form stable homo-oligomers. Presumably, two acetylcholine-binding sites and hence two α -chains per receptor are optimal, and stable homo-oligomers of α would be wasteful. Also, if there were only three types of subunits to form a pentameric ring, say α , β and γ , then one of them would necessarily have complementary

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Mass extinctions

Confusion at the boundary

Stephen K. Donovan

It is fashionable to examine geological boundary sections in search of geochemical anomalies, such as the iridium peak of possible extraterrestrial origin that was discovered at the Cretaceous/Tertiary boundary¹. Most of the boundaries that have been investigated are associated with mass-extinction events (see my recent News and Views article²), but there is one horizon that has excited particular interest. This is the Precambrian/Cambrian (PC/E) boundary, at about 570 million years ago, that immediately preceded the great radiation of the first shelly faunas following the genesis of metazoan organisms in the late Precambrian. It has been suggested variously that this boundary does³ or does not^{4,5} represent a mass extinction. There are two problems in such discussions: the actual level of the boundary has not been defined internationally; and, more seriously, a mass extinction can be recognized only if a

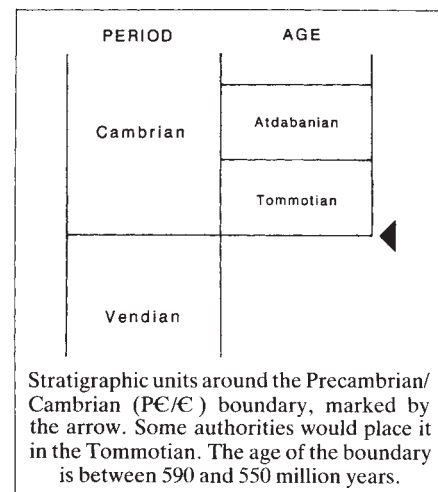
significant faunal turnover is detected, which is difficult in strata where fossils are already so sparse. A new compilation of data⁶ by Crimes, concerning faunal activity around the PC/E boundary, suggests that metazoan behaviour was little affected by any faunal turnover.

There are essentially two forms of fossil, body and trace fossils. As the name implies, a body fossil comprises the remains of the body of an organism, such as the shell of a mollusc or the skeleton of a dinosaur, and almost invariably comprises only the hard, skeletal parts of an organism, although soft tissues and soft-bodied organisms have been preserved in exceptional circumstances. In contrast, trace fossils represent the activities of organisms: tracks, trails, burrows, borings, fecal material are all examples, but the identities of trace-producing organisms are usually impossible to ascertain. Many traces were formed by soft-bodied organisms that are not otherwise preserved. Additionally, one organism may produce different traces, and a single trace morphology may be common to several forming organisms. Many marine traces, however, have a form influenced by the environment, so although the fauna has changed drastically since the PC/E, many trace fossils are essentially unaltered.

Geochemical evidence suggests that various significant events related to organic productivity occurred close to the PC/E boundary. But different rock sequences give different patterns of geochemical fluctuation. Tucker⁷ found a $\delta^{13}\text{C}$ carbon-isotope peak just below the level of the first trilobites in Morocco. This peak could indicate an increase in organic productivity corresponding to the early Cambrian radiation of metazoans, associated with increased plankton diversity, which could approximately correspond to the Tommotian/Atdabanian boundary (see figure).

Magaritz *et al.*⁸, however, found a $\delta^{13}\text{C}$ peak immediately below the Vendian/Tommotian (= PC/E?) boundary in Siberia, followed by a sharp decrease at the boundary and a second, smaller peak early in the Tommotian. The decline between peaks could indicate a decrease in organic productivity, if not an actual extinction event. Hsü *et al.*³ suggested that an iridium anomaly associated with a sharp decrease in $\delta^{13}\text{C}$ found in the Tommotian or Atdabanian of China could be related to a bolide impact and mass extinction.

Others^{4,5} have pointed out that the iridium peak occurs during a period in



which the organisms that produced small shelly fossils were radiating, before the appearance of the trilobites. By this time, the late Precambrian soft-bodied fauna had gone into decline⁹. The new work of Crimes shows that other soft-bodied organisms left a good fossil record across the PC/E boundary in the form of plentiful trace fossils at several localities⁶. Some groups of trace fossils are limited to the late Precambrian but many originated in the late Vendian and in the Tommotian, and continued through most or all of the Phanerozoic⁶. The general pattern is undoubtedly one of increasingly diverse metazoan behaviour (presumably indicating a related radiation of the metazoans) from the late Precambrian and into the early Cambrian, before the appearance of the trilobites. Indeed, the first trilobite trace fossils pre-date the earliest trilobite body fossils, suggesting that soft-bodied trilobites arose very early in the Cambrian or even in the late Precambrian.

Thus, the earliest trace fossils, which record the diversification of the primitive metazoans as a function of their increasingly varied behaviour, show a similar pattern of increase across the PC/E boundary to the small shelly fossils. Whatever we read into the geochemical anomalies, our interpretation of mass extinction must depend upon changes seen in the fossil record. Both body and trace fossils, as well as carbon isotopes, show a pattern of distribution across the PC/E boundary that suggests the early metazoan radiation was not punctuated by the hiccup of an extinction event. □

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FIRST OBSERVATIONS ON A LIVING COELACANTH

READERS of the paper by H. Fricke *et al.* on locomotion of the coelacanth *Latimeria chalumnae* (page 331 of this issue) may be interested in this dramatic account by J. Millot of his attempts to obtain a living coelacanth for investigation.

Throughout the night — which the delighted population of Mutsamudu passed in singing and dancing to celebrate the capture — the Coelacanth was watched over with admirable care. It seemed, although quite bewildered at the sequel to its ascent to the surface, to be taking the situation very well, swimming slowly by curious rotating movements of its pectoral fins, while the second dorsal and anal, likewise very mobile, served together with the tail as a rudder.

After daybreak it became apparent that the light, and above all the sun itself, was upsetting the animal very much, so several tent canvases were put over the boat to serve as some kind of protection. But despite this precaution and the more or less constant renewal of the water, the fish began to show more and more obvious signs of distress, seeking to conceal itself in the darkest corners of the whaler.

At 14.45 hr. it was still swimming feebly; but at 15.30 hr. it had its belly in the air and only the fins and gill-covers were making agonized movements. It was then covered with a sheet and taken immediately to the hospital. There was not a scratch on it, apart from a tiny incision in the centre of the anterior part of the floor of the mouth made by the fisherman when recovering his hook.

It measured 1.42 m. in length and weighed 41 kgm. Chemical and histological investigations could be made under the best possible conditions on perfectly fresh tissues.

From *Nature* **175**, 362; 26 February 1955.

Evolutionary biology

What maintains the frequencies of human genetic diseases?

Jerome I. Rotter and Jared M. Diamond

EVOLUTIONARY biologists can readily understand the persistence of infectious diseases, which represent just one more case of a struggle between two species, in this case the host and the pathogen. But what hinders natural selection from eliminating the genes for inherited diseases, by which we destroy ourselves? In an increasing number of cases, the frequencies of such genes seem to be maintained by compensating advantages¹⁻¹⁰.

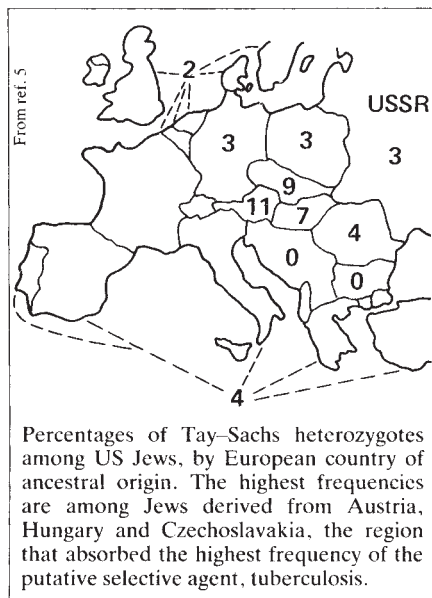
Of course, some genetic diseases occur at frequencies so low that new mutants suffice to replace carriers of the previous generation, who die without reproducing. Duchenne-type muscular dystrophy, and the dominant skeletal malformations achondroplasia and acrocephalosyndactyly are among such conditions thought to be maintained by mutation rates alone¹. The highest known mutation rate for a human genetic disease (that for Duchenne muscular dystrophy) is of the order of 10^{-4} , and most rates are much lower (about 10^{-7}). Thus, mutation can sustain lethal genes of high penetrance only at low frequencies. The founder effect can transiently raise deleterious genes to higher frequencies in local populations of recent origin, as we discussed² in our recent News and Views article. But neither mutation nor the founder effect can explain geographically widespread deleterious genes present at high frequencies in large, old populations — that is, the most familiar diseases with a genetic basis, such as cystic fibrosis, peptic ulcers and diabetes mellitus. These are the genes for which a compensating advantage is suspected.

The classic, and still the only well-understood, example of such compensation is provided by the gene encoding sickle-cell anaemia. The homozygous carriers of this gene die or fail to reproduce because of anaemia, but the much more numerous heterozygous carriers resist malaria better than normal individuals. Thus, the gene is perpetuated in malarial regions by one set of individuals who reap the benefits, while another set pays the price. Similar compensation can sustain other autosomal recessive genes, such as that encoding congenital adrenal hyperplasia (which in the heterozygote form appears to protect Yupik Eskimos against *Haemophilus influenzae* B infections)⁴ or that for Tay-Sachs disease^{5,6}.

The last gene involves a lysosomal enzyme defect (hexosaminidase A), which causes a lipid-storage disease fatal to homozygotes in early childhood. Yet

the Tay-Sachs gene reaches heterozygote frequencies, in some populations of Ashkenazi (eastern European) Jews, of up to 11 per cent⁴. Two other autosomal recessive diseases that involve lipid storage and are either lethal or highly deleterious also reach high frequencies in Ashkenazi Jews: Niemann-Pick disease and adult-type Gaucher's disease, with heterozygote frequencies around 1 and 3 per cent, respectively⁶, involving defects in two other lysosomal enzymes, sphingomyelinase and glucocerebrosidase, respectively. Thus, the same human population has independently evolved three mechanisms for lysosomal lipid storage, suggesting some shared benefit to the heterozygotes.

In the case of Tay-Sachs disease,



several types of evidence suggest this benefit to be resistance to tuberculosis, which acted as a stronger selective force on Ashkenazi Jews than on other ethnic groups, presumably because Jews were confined to crowded town environments while the town populations of other ethnic groups were subsidized by immigration from the countryside⁵. Ashkenazi Jews reported a lower frequency of tuberculosis deaths than non-Jewish populations from the same area. Families of Tay-Sachs patients reported a lower frequency of deaths than control Jewish families. The highest frequency of the Tay-Sachs gene in Ashkenazi Jews was in the area (Austria-Hungary) that had the highest incidence of tuberculosis, the putative selective agent (see figure)⁵. Hence,

deaths from lipid storage diseases may have been the price that Tay-Sachs (and Niemann-Pick and Gaucher?) homozygotes paid so that the more numerous heterozygotes could survive.

A different distribution of compensation may be operating in idiopathic haemochromatosis (IHC), such that men suffer and women benefit⁷. Like sickle-cell anaemia and the three lipid-storage diseases discussed above, IHC is an autosomal recessive disease: with a heterozygote frequency of 10 per cent, it is probably the commonest abnormal gene identified in the United States and Europe to date. The abnormality consists of increased rates of intestinal iron absorption, but build-up of iron stores is still sufficiently slow that they do not reach toxic levels until late in life, and then mainly in people with a high dietary iron intake. Furthermore, serious symptoms are 5–10 times more frequent in men than women, as women routinely experience high iron losses through menstrual bleeding, pregnancy and lactation. That the IHC gene is considered deleterious at all reflects a male-chauvinist evolutionary view: increased iron absorption is good for most women. From an unbiased evolutionary view, the deaths of some post-reproductive males are a small price to pay for protecting many women in the same population against anaemia.

Although IHC benefits women, and sickle-cell anaemia and the lipid-storage diseases can benefit heterozygotes of either sex, these diseases still resemble each other in that benefits and costs accrue to different individuals. It remains to consider diseases where the individual who bears the risks may also reap the benefits that can occur in one of three forms: resistance to infectious diseases; improved prenatal survival; or improved postnatal survival under particular environmental circumstances.

Resistance to tuberculosis, and possibly to cholera and typhoid, could be a benefit of genes predisposing to the ulcers resulting from high pepsinogen I levels and high rates of gastric-acid secretion⁷. Hydrochloric acid in the stomach is a barrier against bacteria ingested by mouth or else redistributed from the lungs by coughing and swallowing. Individuals with low rates of gastric-acid secretion are most prone to tuberculosis, cholera and typhoid. In contrast, Scotland has the distinction of the highest frequencies of acid-hypersecretion-linked ulcers and (formerly) of the putative selective agent, tuberculosis. The steep rise in the frequency of this disease during the crowding of the industrial revolution may have propelled ulcers to high frequency, as a post-reproductive price for surviving tuberculosis in pre-reproductive years.

Improved prenatal survival seems to be the main benefit of genes encoding

insulin-dependent diabetes mellitus (IDDM), which was generally fatal until the advent of insulin replacement therapy⁸. In affected families, normal fetuses are more likely to miscarry than those with diabetes-linked HLA antigens. Because only 20 per cent of newborns with the IDDM diabetogenic genotype actually become diabetic, 80 per cent of the carriers reap the prenatal benefits without paying the postnatal price.

As for non-insulin-dependent diabetes mellitus (NIDDM), its expression in several societies (Yemenite Jews, Pima Indians, Micronesians) reached high levels within a few decades after those people shifted from spartan subsistence conditions to a westernized diet⁹. This suggests that NIDDM diabetogenic genes are beneficial under the former conditions but detrimental under the latter¹⁰. In fact, diabetic mice survive starvation better than normal mice, perhaps because their overactive insulin-mediated glucose metabolism that leads to diabetes on a high-carbohydrate diet permits them to lay down glycogen reserves against starvation when experiencing a limited or intermittent food intake. Hence it would be interesting to compare the frequency of NIDDM in descendants of Irish people who survived the potato famine with those who escaped by emigrating. We would anticipate a higher frequency of the disease today in Ireland itself than in the emigrants, if starvation favoured diabetic humans as it does diabetic mice. Other genetically influenced diseases of westernized societies, such as hypertension on a high-salt diet and certain familial hyperlipidaemias, similarly may arise from genes formerly advantageous to peoples with spartan diets.

Thus, in the examples we have discussed here, evolution may favour genes that penalize homozygotes, men, adults or people with certain lifestyles, because those genes benefit heterozygotes, women, fetuses or people with other lifestyles. Those of us suffering from genetic diseases are victims of a tragic faustian bargain in which natural selection plays the role of Mephistopheles. □

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Site-specific recombination

Making Holliday junctions

David Leach

IN 1964, Robin Holliday suggested¹ a model for homologous recombination of DNA in which two pairs of symmetrical strand exchanges are made to form and resolve an intermediate which has since been named a Holliday junction (Fig. 1). Kitts and Nash, in their paper on page 346 of this issue², and Nunes-Düby, Matsumoto and Landy³, now report evidence for such a mechanism in the recombination reaction used by bacteriophage lambda to insert and excise its genome from that of its host *Escherichia coli*. This is the first recombination reaction for which such a mechanism has been demonstrated.

Campbell's model⁴ for bacteriophage lambda integration via reciprocal exchange at specific sites on the phage and

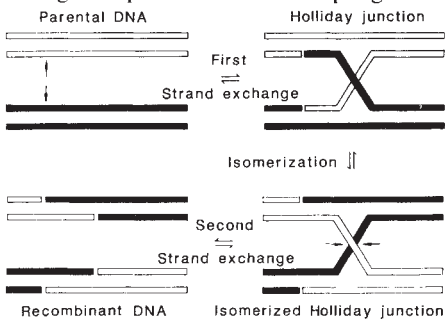


Fig. 1 Crossing over of DNA strands by the Holliday model. DNA strands of the same polarity are nicked at identical positions and exchanged to generate a Holliday junction. This is then resolved by a second pair of strand exchanges. One possibility is that isomerization between two forms of the Holliday junction can occur and that resolution of only one of these leads to crossing over. Arrows show sites of strand exchange required for crossing over.

bacterial chromosomes is widely accepted and considerable insight has been gained on the nature of the proteins and DNA sequences involved in this reaction. Integration occurs by site-specific recombination between a 240-base pair (bp) segment of phage DNA (*attP*) and a 25-bp segment of chromosomal DNA (*attB*). These segments share 15 bp of homologous DNA (the core region) and it is in this region that strand exchange occurs between precisely defined nucleotides separated by 7 bp. The product of integration is a lambda prophage flanked by two hybrid sites, *attL* and *attR*, which, on excision, regenerate *attP* and *attB* (Fig. 2). An interesting feature of this reaction is the strict requirement for homology in the 7-bp overlap region. Single base-pair differences between *attP* and *attB* in this region can drastically reduce recombination. Such changes from the canonical (correct) sequence are called *saf* (site-

affinity) mutations. It is the homology rather than the precise sequence of the 7-bp regions that is important as recombination is restored if both participating sites carry the same *saf* mutation⁵. This requirement for homology in a site-specific reaction could not have been predicted *a priori* and reflects an important feature of the recombination mechanism.

The observation that homology is required in the overlap region, and that a characteristic +2 change in linking number (see Fig. 3) occurs on *in vitro* recombination of a partially supercoiled inversion substrate, led to the attractive hypothesis^{6,7} that synapsis of *attP* and *attB* occurs via a four-stranded DNA intermediate followed by strand exchange associated with precisely defined strand rotation. Strand exchange might occur by simultaneous cleavage at the four points shown in Fig. 2, rotation and rejoining; or one pair of strand-exchange reactions might precede the other to generate and resolve a Holliday junction.

It is this second possibility that has now been conclusively demonstrated. Nunes-Düby, Matsumoto and Landy³ find that a nick leaving a 5' phosphate group at the site of exchange to the left of the 7-bp overlap blocks the formation of any intermediates, whereas a similar nick at the site of exchange to its right leads to the accumulation of Holliday junctions in both integrative and excisive recombination. Similarly, *saf* mutations in the left 3 bp of the overlap region block the formation of intermediates, whereas *saf* mutations in the right 4 bp lead to the accumulation of Holliday junctions^{2,3}. As shown by the work of Kitts and Nash in this issue², the latter reaction is stimulated by the substitution of thiophosphates for

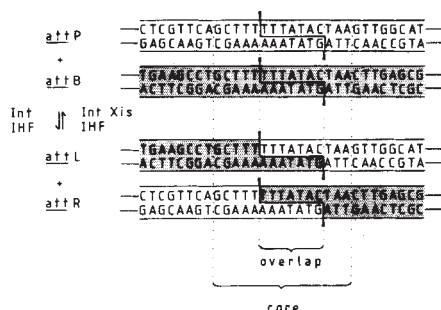


Fig. 2 Core and overlap regions of the lambda *att* sites. Positions of strand exchange are indicated by darts. The integration reaction, mediated by the phage-encoded protein integrase (Int) and the host-encoded complex integration host factor (IHF), generates *attL* and *attR* from *attP* and *attB*, while the excision reaction to form *attP* and *attB* from *attL* and *attR* also requires the phage-encoded protein excise (Xis).

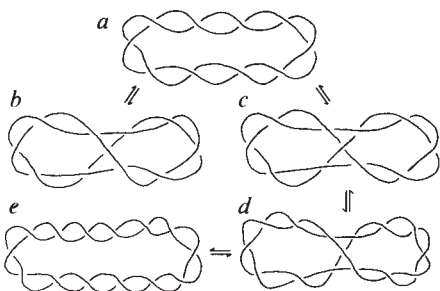


Fig. 3 Effect of isomerization on the linking number of a simple inversion substrate with no supercoils trapped between the sites of exchange (a). Two different forms of the Holliday intermediate (b, c) can theoretically be formed by the first strand exchange. The consequence of isomerization of one of these (c) has been shown. This generates (d) which can be resolved to give (e). The pathway (a-c-d-e) is an alternative way of looking at what in terms of the four-stranded model would be described as Wilson pairing followed by -90° rotations⁹, and leads to an unknotted product with a +2 change in linking number when no net supercoils are trapped between the sites and to a +3 trefoil knot when two negative supercoil nodes are trapped. Supercoiling of the DNA^{9,10} is omitted from the figure for clarity. The molecule in (a) has five helical turns and that in (e) has seven (a +2 change in linking number). In negatively supercoiled DNA, this change will result in relaxation of supercoiling.

phosphates in the bottom strand of *attB*, as drawn in Fig. 2.

The frequency of formation of Holliday junctions argues that they are intermediates in the normal reaction, which both groups^{2,3} conclude occurs via two pairs of strand exchanges. The first, to the left of the overlap, generates a Holliday junction, and the second, to its right, resolves the structure to the recombinant products. Efficient integrase-mediated resolution of synthetic *att*-containing Holliday junctions has previously been demonstrated⁶. The fact that right-hand side *saf* mutations in *attB* do not inhibit the formation of Holliday junctions but do inhibit their resolution, demonstrates that homology in the overlap region is involved in processes other than synapsis. Four-stranded DNA models where the only role of homology is for synapsis are therefore excluded.

Both groups^{2,3} agree that homology may be required to allow branch migration of the Holliday junction from the position of the first exchange to that of the second. The lack of evidence for four-stranded DNA in this and earlier work⁹ does not remove the requirement for defined rotation of strands to generate the observed topology of the products. The analysis developed for the four-stranded DNA models remains relevant in this respect, and one of the next tasks is to define and explain the precise movement of strands with respect to each other.

The concepts of paired single-strand exchanges to form and resolve Holliday junctions and the ability of Holliday

junctions to branch-migrate were first developed for homologous recombination, as was the concept of isomerization (Fig. 1). Could it also be usefully applied to site-specific recombination? Isomerization involves the rotation of DNA strands to interchange crossing and non-crossing strands of a Holliday junction if the strands are not free to adopt a symmetrical (tetrahedral) conformation. Perhaps isomerization can be viewed as the reaction determining the nature of strand rotation associated with recombination (Fig. 3). □

Nuclear structure

High spins stretch the limit

J.D. Garrett

OCCASIONALLY, a singular discovery produces a renaissance in a specific scientific discipline; supernova 1987A and the new high-temperature superconductors spring to mind. The discovery last year of rapidly rotating, superdeformed nuclei¹, which I discussed in a News and Views article², may not have been front-page news, but it has reinvigorated the study of nuclear structure. The discovery represents more than simply yet another supersomething. It provides a detailed view of a complicated physical system in an extreme condition. The nucleus in question, dysprosium-152 (¹⁵²Dy), has been scrutinized by many groups. Recent lifetime measurements³ confirm the superdeformed shape, and W.J. Swiatecki has suggested⁴ that the nucleus shows a transition from a liquid-like to a solid-like state as its rotation increases.

The strong, short-ranged, nuclear interaction between the consistent nucleons of a nucleus leads to a stability condition that maximizes the number of neighbouring nucleons. Nucleons inside the nucleus are more strongly bound than those on the surface, so that we expect all nuclei to be spherical. But other factors must be considered. Paradoxically, the mean free path of the nucleons in the nucleus is sufficiently long (a result of the Pauli principle that allows only one nucleon per quantum state) even in the presence of the strong nuclear interaction for nucleons to arrange their motion in a quantum-mechanical shell structure. This shell structure (similar to the electronic shell structure of atoms), also affects the equilibrium nuclear shape, and most nuclei are ellipsoidal, not spherical. Electrostatic repulsion between the protons also promotes nonspherical shapes, the protons being further apart on average, in an ellipsoid. This effect, which increases with increasing charge of the nucleus is responsible for fission of the heaviest nuclides.

Twenty years ago, Strutinsky⁵ developed a technique for calculating the

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energy associated with the shell effect, allowing realistic predictions of nuclear shapes. He ascribed the observed⁶ isomeric (delayed) fission of several actinide nuclei to stable, highly deformed shapes, with the ratio of major to minor axes (*a/b*) of 2, termed superdeformed. He next predicted⁷ that shell corrections should increase the stability of various lighter nuclei. Such cases were neither expected nor observed as fission isomers, the electrostatic effects being too small to give the required added stability at such large deformations. But the effect of the centrifugal distortion on the shape of a rotating nucleus is predicted to increase the stability of rapidly rotating superdeformed nuclei⁸.

These predictions were confirmed with the discovery last year¹ of a sequence of superdeformed states of ¹⁵²Dy with *a/b* ~ 2. One per cent of the high-spin nuclei are in the superdeformed state, the rest being in the normal-deformed state (the two are separated by a potential barrier). This may not seem to be a large proportion, but it is 100-fold larger than that found with fission isomers and makes possible detailed studies of nuclei on the verge of fission and centrifugal instabilities. The quadrupole moment of ¹⁵²Dy, determined³ from the lifetimes ($\sim 10^{-14}$ s) of the rotational states, is $(19 \pm 3) \times 10^{-24}$ e cm², where *e* is the elementary unit of charge. This is much larger than in normal nuclei (*a/b* ~ 1.25) of similar size, which confirms the superdeformed shape of ¹⁵²Dy.

One of the most surprising features of the superdeformed state in ¹⁵²Dy is the precision with which its rotation can be described. For example, the moment of inertia over the range of angular momentum *I* = 22–60 can be reproduced to the 0.05 per cent precision of the experimental measurements by a simple mathematical expression⁴. Such an analysis also indicates that while the stiffness of the nuclear potential with respect to changes in the moment of inertia varies by more than an order of magnitude between *I* = 24

and 46, this quantity stabilizes at a nearly constant value for $I \geq 48$. Swiatecki¹ describes this behaviour as a "hint of centrifugal solidification" at the largest angular momenta. That is, at these angular momenta the nucleus stretches with the nucleons remaining in their original states rather than being redistributed adiabatically into the lowest energy state.

¹⁵²Dy nuclei with angular momentum $I > 28$ seem to prefer being in the superdeformed state, but below this the γ -ray transitions lead predominantly to the normal deformed state. By $I=24$ the superdeformed states are almost completely vacant. No calculations predict such an abrupt disappearance of the stable superdeformed shape. This feature may be associated⁸ with pairing, another property of nuclei. Because of the strong nuclear force and the quantum-mechanical Pauli principle, nucleons prefer to arrange themselves two by two in orbits with the partners moving in opposite directions. The consequences of nuclear pairing include nuclear superfluidity, the quasiparticle spectrum of nuclear states and special selection rules for pair transfer.

The decay from super to normal deformation can be considered as a penetration, enhanced by the pairing^{9,10}, of the potential-energy barrier that separates the two structures. Coriolis and centrifugal forces in a rotating nucleus are predicted¹¹ and have been observed¹² to quench pair correlations, just as the magnetic fields quench electron pairing in supercon-

ductors. The de-population of the superdeformed configuration at $I < 28$ can then be attributed⁹ to the onset of pair correlations, with its associated enhanced barrier penetration.

Investigations of high-spin superdeformation have begun at nearly all suitable facilities, but no other similarly deformed nuclei have yet been reported, although rumours abound. Deformations intermediate to normal deformed nuclei and superdeformed ¹⁵²Dy, however, have been reported for cerium¹³, neodymium^{14,15}, praseodymium¹⁵, and zirconium¹⁶ isotopes, yielding further information about the shell structure in rotating deformed nuclei. The idea that added stability applies only for $a/b \sim 1.25$ (normal deformation) and 2 (superdeformation) is apparently too simple. □

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Biological diversity

What makes a forest rich?

Peter D. Moore

As soon as biologists became involved in voyages of discovery in the eighteenth and nineteenth centuries, they were struck by the global patterns of vegetation and animal communities and the ways in which some areas can support a greater diversity of life than others. This relates directly to the number of organisms that can make a living out of the available resources, which in turn may be dependent on the extent of these resources, the limitations of the physical environment, the ways in which resources present themselves for exploitation (habitat heterogeneity), the number of species geographically available, site accessibility, and so on. There have been many attempts to find out which factors are most important in producing species richness, but quantitative analysis has been difficult. Elsewhere in this issue (*Nature* **329**, 326–327; 1987), D.J. Currie and V. Paquin report their analysis of trees throughout North America, and identify the most influential variable that determines the species richness of forests there.

In the past, most research has been concentrated on the influence of latitude and isolation in controlling biological diversity, together with the effects of major climate and environmental disturbances such as the onset of glaciation. There is evidence for a general decline in richness of species from the tropics to the tundra, creating an overall latitudinal gradient in diversity; isolated locations (from peninsulas to islands) also exhibit declining species richness. In Europe, the depauperate state of the tree flora has been related to the generally east–west arrangement of the main mountain systems of this continent, the Pyrenees and Alps; these cut off the migration routes of tree species moving south as climates cooled and may have led to the extinction of some taxa that survived in the New World because of the generally north–south alignment of mountain chains there (the Rockies and the Appalachians).

However, this picture is complicated by factors such as habitat diversity, for the

requirements of more species can be satisfied if a site is topographically or ecologically variable. When investigating frogs in Amazonia, B.L. Zimmerman and R.O. Bierregaard (*J. Biogeogr.* **13**, 133–143; 1986), found the richest sites to be those which contained the mud wallows of the collared peccary, thus supplying the precise needs of breeding frogs. So generalizations about the causes of diversity may well be difficult to construct.

There is also the practical problem of taxonomy. Almost all studies of species richness concentrate, very naturally, on just one taxonomic group—frogs, lizards, birds, bats, flowering plants, and so on. But each group, like the frogs mentioned above, may have some peculiar requirement of its own that obscures the general causes of diversity. In the case of the study by Currie and Paquin reported in this issue, the raw data relate to trees. Dividing the whole of the United States and Canada into 336 quadrats, these authors map the occurrence of all 620 native tree species and draw contours of richness (see their Fig. 1 on page 326). It is immediately obvious that the tree data do not follow any simple pattern. There are no strong latitudinal gradients, especially in the west, and the areas with the highest richness are clearly concentrated in the southeast. The results do not tally with bird data, for rich and poor areas do not coincide, nor are trees noticeably less rich on peninsulas.

Currie and Paquin tested their correlations with several variables using non-linear regression, and find the strongest relationship is between richness and annual evapotranspiration. For trees, it seems, dry spots are bad news. Once the authors allowed for this factor, high richness turned out to be associated with quadrats in which altitudinal variability is high—the habitat diversity effect again.

On a global scale, evapotranspiration is a major factor influencing primary productivity, so the results of Currie and Paquin imply that the richness of forest trees is essentially determined by potential productivity of an area. A further implication is that historical factors, such as the intensity or frequency of glaciation and problems of migration and immigration, are of lesser significance in controlling contemporary richness. By using their climatic model to predict the forest richness of the British Isles (and coming very close to the mark) Currie and Paquin show that Europeans cannot use their unfortunate experiences during the Pleistocene as an excuse for present-day botanical poverty; rather, it should be put down to climate. Forest richness, it seems, is mainly the product of the resource base—water, warmth and solar energy. □

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Detection of global carbon dioxide effects

SIR—The quest for clear evidence of effects of global carbon dioxide (CO_2) has been a high priority for a number of years. Climatologists have been diligently searching for signs of the worldwide warming predicted by various numerical models of the CO_2 greenhouse effect, while others have looked for the photosynthetic stimulation of the biosphere of which there have been experimental demonstrations.

Although most responsible climatologists are careful to say that the empirical climatic evidence, while consistent with their predictions, is by no means proof of them, there have been several valid claims of detection of a biological signal, until recently all based on the discrimination of an increasing amplitude in the seasonal CO_2 cycle, usually in the Mauna Loa, Hawaii, record, but also in a few other datasets. Now, however, a published study (*J. geophys. Res.* **92**, 5497; 1987) by I.G. Enting presents an important new piece of evidence for the reality of global photosynthetic stimulation due to the rising atmospheric CO_2 concentration.

Enting has analysed this interannual variation in the seasonal cycle of CO_2 concentration at Mauna Loa, verifying the tendency toward larger amplitudes in recent years and finding a strong correlation between amplitudes of spring peaks and the fall troughs that follow. But there is correlation between amplitudes of fall troughs and the following spring peaks.

After eliminating several other possible mechanisms, including fossil carbon release, ocean-carbon exchange and various atmospheric transport processes, Enting notes that, "of all the processes that significantly influence atmospheric carbon dioxide, only biotic processes seem to be capable of providing the requisite seasonal asymmetry". His interpretation of the spring-peak/fall-trough correlation, together with the fall-trough/spring-peak non-correlation, is that "the interannual variation in the amplitude of the seasonal cycle is associated with variations in individual summer growth seasons and that the carbon that is assimilated into the biota is released to the atmosphere over several years", which we know to be true.

The case for global CO_2 effects on worldwide vegetative productivity would therefore appear to be firm. We know, for instance, that the terrestrial biota is responsible for the seasonal cycle itself and that the amplification of the cycle with time appears to be explicable only in terms of CO_2 -induced stimulation of photosynthetic activity. Now, it also appears that a unique asymmetry in the interannual variation in the seasonal cycle is also

explicable only in terms of photosynthetic variations. Hence, we appear to have little recourse but to acknowledge the reality of this ubiquitous phenomenon, as many have already done. Indeed, as Morison (*Nature* **327**, 566; 1987) has recently noted with respect to a number of these studies, they emphasize "that the global rise in CO_2 is already having important effects on the biosphere".

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Detection of sickle cell anaemia and thalassaemias

SIR—Prenatal diagnosis for sickle cell anaemia and α -thalassaemia, first introduced^{1,2} in 1975, now depends on the analysis of DNA, which is acquired either from fetal cells safely by amniocentesis at week 15 of gestation, or at week 7–10 by chorionic villus biopsy^{3,4}. The chief impediment to widespread prenatal diagnosis has been that current techniques, based on Southern blot analysis or dot blot hybridization with radioactive globin-gene or oligonucleotide probes, are complicated and difficult to implement. We hope that the technique we describe here, which is simple, rapid and does not require radioactive isotopes, will be particularly useful in many of the countries where these two disorders are common.

The method is based on the recently described procedure for amplifying DNA *in vitro* by what is known as the polymerase chain reaction (PCR)^{5,6}. By using the DNA polymerase from the thermophilic bacterium *Thermus aquaticus*⁷, DNA synthesis can be performed at a high temperature, which so increases the specificity of the oligonucleotide priming that the amplified sequence can be directly visualized after staining as a discrete band on gel electrophoresis, circumventing the need

for DNA hybridization or radioactive probes.

The usual mutation responsible for the severe form of α -thalassaemia, homozygosity for which results in the lethal condition of hydrops foetalis⁹, is a deletion of approximately 23 kilobases of DNA within the α -globin gene cluster¹⁰. To detect this deletion, we primed DNA synthesis in the PCR with a pair of oligonucleotides that produce amplification of a 136-base-pair (bp) region of the α -globin gene cluster between the $\psi\alpha$ and $\alpha 2$ -globin genes. This region was chosen because it lies outside the repeat regions¹¹ of a α -globin gene cluster and its amplification yields low background. We also added to the reaction mixture two primers that amplify a 110-bp segment of the β -globin gene, which served as a control. In amplified DNA from individuals with the Southeast Asian form of homozygous α -thalassaemia, only the control 110-bp β -globin gene fragment is detected, whereas in normal individuals both this and the 136-bp α -globin gene cluster fragments are visualized (Fig. 1).

To detect the sickle cell mutation, we amplified a 294-bp segment of the β -globin gene which extends from nucleotide -36 through the first exon to nucleotide 117 of the first intervening sequence. (The two primers used to amplify this segment will not amplify the δ -globin gene.) After amplification, the DNA fragments were digested with a restriction endonuclease (*Oxa*NI) that has a recognition site which is abolished by the A→T mutation at codon 6 in the sickle gene^{12,13}. Thus, the segment derived from normal DNA is cleaved into two fragments of 191- and 103-bp, while the 294-bp fragment amplified from the DNA of a sickle cell anaemia patient remains uncleaved. In the heterozygote, all three fragments are visualized. These fragments can be detected either by ethidium bromide staining (Fig. 2, left), which requires visualization

Fig. 1 Polyacrylamide gel electrophoresis of amplified DNA from normal control (N) and two fetuses with hydrops foetalis (Hy). The amplified α -globin gene cluster fragment was 136 bp in length and the β -globin gene fragment 110 bp.

Methods Genomic DNA was isolated from white blood cells and amniotic cells¹⁴. The polymerase chain reaction was performed using a protocol modified from ref. 8 and New England Biolabs. Fifty picomoles of each pair of oligonucleotide primers was mixed with 1 μg of genomic DNA in a 100- μl volume reaction mixture according to the suggestions of the manufacturer (New England Biolabs). The mixture was incubated at 95°C for 5 min to separate the DNA strands, spun in a desktop centrifuge for 10 s and 2–3 units of *Thermus aquaticus* DNA polymerase were added. The mixture was sealed under 35 μl of mineral oil and incubated first at 63°C for 45 s, then at 93°C for 30 s, and then 50°C for 30 s. This heating cycle was repeated 30 times before reducing the temperature to 4°C for storage. Ten- μl aliquots from the amplification mixture were loaded on a 10×10×0.1 cm 12% polyacrylamide gel in 1× Tris-borate, pH 8, and run at 45 mA for 45 min. The gel was stained with 1 $\mu\text{g}/\text{ml}$ ethidium bromide for 5 min and the DNA bands visualized under ultraviolet light. The synthetic oligonucleotide primers used to amplify a segment of the α -globin gene cluster were 5'-TACTGTAGATACCCGTGTACAA-3' and 5'-ATCATGGAAACATAGTAAT-3'. The sequences of the primers for amplification of the β -globin gene were 5'-ACACAACTGTGTTCACTAGC-3' and 5'-CAACTTCATCCACGTTCCACC-3'.



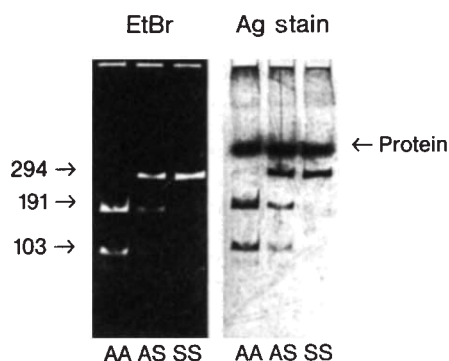


Fig. 2 Polyacrylamide gel electrophoresis of the *Oxa*NI-digested amplified DNA from normal control (AA), sickle cell trait (AS) and sickle cell anaemia (SS). The two primers used were 5'-GGGCTGGGCATATAAAGTCA-3' and 5'-AATAGACCAATAGGCAGAG-3'. DNA amplification as in Fig. 1, followed by digestion of a 10- μ l aliquot of the reaction mixture with 10 units of *Oxa*NI in a 20- μ l mixture for 1 h at 37°C according to the manufacturer's conditions. Two μ l of 0.25% bromophenol blue was then added to the digestion mixture and electrophoresis was performed as in Fig. 1, after which the same gel was stained with ethidium bromide (EtBr) or silver (Ag) stain. The latter also stains a protein band.

with ultraviolet light in a darkroom, or by silver staining¹⁴, which enables the DNA bands to be seen directly and with the same degree of sensitivity as the ethidium bromide stain (Fig. 2, right).

A major advantage of this method is its simplicity. We have performed the polymerase chain reaction on lysed chorionic villus samples without prior DNA extraction⁸, and by initiating amplification on lysed cells according to Saiki *et al.*⁶. Thus, the prenatal diagnosis procedure is reduced to the following steps. Fetal cells are lysed and the appropriate polymerase chain reaction performed for 30 cycles, which can be easily accomplished by incubating the tubes sequentially in three water baths at the desired temperatures. For α -thalassaemia, an aliquot of the reaction is immediately fractionated by gel electrophoresis, while for sickle cell anaemia an aliquot is first digested for one hour with *Oxa*NI. This part of the test is no more difficult than routine haemoglobin electrophoresis. After staining the gels, the mutations can be directly detected. Diagnosis can be completed in 3 to 4 hours.

Both specific amplification and direct visualization of polymorphic DNA sequences have been used for linkage analysis and for carrier detection and prenatal diagnosis of haemophilia A⁸. Our data show that this simple non-radioactive method can readily and directly diagnose genetic disorders that are due to gene deletions or to mutations which alter restriction endonuclease cleavage sites.

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Visna and myelin basic protein

SIR—The demyelination caused by visna virus in sheep has long been considered to be one of the best animal models for multiple sclerosis, but the mechanism for the persistence and slow rate of tissue damage remains obscure¹.

While we were searching known protein sequences in the Protein Identification Resource (National Biomedical Research Foundation) for homologies between an unusually hydrophobic peptide present in sheep² and the human 21.5 K myelin basic protein³ (MBP), we found the following homology between visna *pol*⁴ and MBP (single-letter code):

MBP SGKVPW-LKPGR
Visna *pol* TGKIPWILLPGR

This region is at the junction of sequences coded by exons 1 and 2 in the MBP gene. As threonine and isoleucine should be functionally similar to serine and valine, the observed homology appears as striking as any reported to support 'molecular mimicry'^{5,6} as a basis for demyelinating diseases. Only about six amino acids are required for an antigenic determinant, so it is likely that an immune response to this region of the visna protein could cross react with MBP. Thus, there is potential for an immunological molecular mimicry.

The homology is even more striking when the messenger RNA sequences are considered. All but one of the 15 nucle-

tides encoding GKIPW of visna *pol* and GKVPW of MPB are identical. Considering that the probability of this alignment occurring at random is 4.3×10^{-8} , visna may have evolved this sequence to mimic the MBP mRNA sequence as a way of disrupting MBP metabolism in sheep oligodendrocytes. This might represent a type of intracellular molecular mimicry that could be a contributing cause of certain characteristics of visna infections, such as persistence and demyelination.

As retroviruses are already being investigated for involvement in multiple sclerosis⁷, our observations might inspire a more specific search for a lentivirus with an ability to mimic MBP sequences in human oligodendrocytes.

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Enzyme identity maintained

SIR—In his recent News and Views¹ article, Roger Pain rightly draws attention to the remarkable discovery² (see also ref. 3) that the β -subunits of the tetrameric ($\alpha\beta$) enzyme prolyl-4-hydroxylase are identical to the polypeptides comprising the dimeric enzyme protein disulphide-isomerase (PDI). In his analysis of this unexpected example of cellular parsimony, however, Pain's emphasis is misplaced. In suggesting that PDI "has been viewed in the wrong light all along" and that its mechanism of action involves a notional affinity for prolyl residues, he ignores extensive evidence on the distribution, properties and active site of PDI.

It has long been known that in most tissues 'inactive' β -subunits of prolyl hydroxylase are in great excess (50-fold in adult mammalian liver) of those found associated with the holoenzyme⁴. Indeed, the question had been raised whether its involvement in the hydroxylase was this polypeptide's primary function⁴.

The recent papers from Kivirikko and colleagues^{2,3} resolve this question; the primary function of these subunits is as PDI. Only in tissues actively synthesizing collagen is a significant proportion recruited by α -subunits to form prolyl-4-hydroxylase tetramers. The accompanying table shows the proportion of cell protein found as PDI dimers and as β -subunits of prolyl-4-hydroxylase tetramers in some secretory tissues. Note that even

Insulin-like growth factor II receptor as a multifunctional binding protein

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The primary structure of human insulin-like growth factor II receptor, predicted from the complementary DNA sequence, reveals a transmembrane receptor molecule with a large extracellular domain made up of fifteen repeat sequences and a small region homologous to the collagen-binding domain of fibronectin. The structural and biochemical features of the IGF-II receptor appear identical to those of the cation-independent mannose-6-phosphate receptor.

THE insulin-like growth factors IGF-I and IGF-II are mitogenic polypeptides with structural and functional homologies to the hormone insulin^{1,2}. *In vitro*, both peptides stimulate a broad range of insulin-like actions, including acute metabolic effects, as well as long-term mitogenic effects^{2,3}. *In vivo*, IGF-I is believed to mediate the growth-promoting actions of growth hormone on skeletal cartilage and other tissues³, perhaps in an autocrine or paracrine fashion⁴⁻⁶. The actions of IGF-II *in vivo* are less clear, but studies of rat IGF-II expression suggest that it has a function in fetal and early neonatal development, possibly also by a paracrine mechanism⁴⁻⁶. The high expression of IGF-II in the brain implies that this peptide is important in the function of the central nervous system⁴⁻⁶.

The physiological actions of IGF-I and IGF-II are mediated by specific interactions with cell surface receptors. Two distinct IGF receptors, the type-I and type-II receptors, have been identified (reviewed in ref. 7). Because type-I and type-II receptors preferentially bind IGF-I and IGF-II, respectively, they are also referred to as the IGF-I and IGF-II receptors. The IGF-I receptor is the more thoroughly characterized. It has a higher affinity for IGF-I than for IGF-II, and binds insulin with moderate affinity. Its structure is closely related to that of the insulin receptor: the receptors for both insulin and IGF-I are disulphide-linked glycoproteins composed of two extracellular α -subunits of relative molecular mass (M_r) 135,000 (135K) and two β -subunits (M_r 95 K) which span the plasma membrane⁷. The α -subunit contains the ligand binding site and a distinctive cysteine-rich region⁸⁻¹⁰; the β -subunit contains a ligand-activated tyrosine kinase domain and autophosphorylation sites. Recent comparisons of the primary structures of the two receptors⁸⁻¹⁰ demonstrate a high degree of similarity in many receptor regions, particularly in the tyrosine kinase domain.

The IGF-II receptor is distinct from the IGF-I and insulin receptors⁷. It has a high affinity for IGF-II, a low affinity for IGF-I, and does not bind insulin^{11,12}. Affinity cross-linking studies with labelled IGF-II (refs 13 and 14), as well as receptor purification studies^{15,16}, show that IGF-II interacts with a protein of M_r 220 K. Under reducing conditions, the apparent M_r of the protein shifts to 250 K–270 K. This behaviour suggests that the conformation of the native monomer is constrained by intrachain disulphide linkages. The receptor appears to be glycosylated, as pretreatment of cells with tunicamycin, an inhibitor of N-linked glycosylation, decreases the apparent M_r of the receptor to 232 K (under reducing conditions)¹⁷. The purified IGF-II receptor does not seem to possess tyrosine kinase activity, although it is phosphorylated on tyrosine residues *in vivo*¹⁸. Insulin stimulates the translocation of IGF-II receptors from

an intracellular compartment to the cell surface¹⁹, perhaps by changing the phosphorylation state of the IGF-II receptor²⁰.

The physiological function of the IGF-II receptor is poorly understood⁷. Because the two IGFs bind to some extent to both IGF receptor types (as well as to the insulin receptor), and because insulin and the IGFs often produce similar biological responses, it has been difficult to determine which responses are mediated by which receptor. Although the IGF-II receptor has the highest affinity for IGF-II, studies using antibodies against these receptors indicate that several IGF-II effects in cell culture systems are mediated by the insulin to IGF-I receptors^{7,12,21-23}. These findings have led to the intriguing question as to whether the IGF-II receptor is actually a transmembrane signalling protein or has some other structural or transport function.

To enhance our understanding of the structure and function of the IGF-II receptor, a complementary DNA encoding the human receptor has been cloned, sequenced, and expressed in *Xenopus laevis* oocytes. The IGF-II receptor was purified from a rat liver cell line, and tryptic peptides obtained from these receptor preparations were sequenced. Corresponding oligonucleotide probes were used to isolate a cDNA coding for the

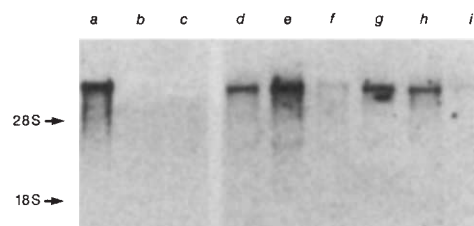


Fig. 1 Analysis of IGF-II receptor transcripts in rat (a–c) and human (d–i) cell lines and tissues. Poly(A)⁺ RNA (a–c, 2 μ g per lane) or total cellular RNA (d–i, 20 μ g per lane) was prepared²⁴ from a, BRL-3A rat liver cells, b, rat liver, c, rat kidney, d, HepG2 hepatoma cells, e, PLC/PRF/5 (Alexander) hepatoma cells, f, Mahlavu hepatoma cells, g, HeLa cells, h, Ewing's sarcoma cells and i, HUT78 T-cell lymphoma cells. RNA was electrophoresed on formaldehyde-containing agarose gels⁵⁴, transferred to nitrocellulose, and hybridized with labelled cDNA inserts from λ phage K3 (a–c) or H5.1 (d–i). cDNA labelling and hybridization were as described in the Fig. 2 legend. The mobilities of 28S and 18S ribosomal RNAs are indicated on the left. A single band of ~9.0 kb was detected in all lanes; the faint lower band in lanes (d–i) is a shadow caused by the large amount of 28S RNA present in these total RNA preparations.

[illegible]

rat receptor. The rat cDNA was then used to isolate human clones making up the 9.1-kilobase (kb) full-length cDNA. Surprisingly, the sequence of the human IGF-II receptor is very similar to the recently determined partial sequence of a bovine mannose-6-phosphate receptor²⁴, a protein believed to be involved in the transport of lysosomal enzymes from the cell surface and Golgi apparatus to lysosomes (reviewed in ref. 25). The properties of the IGF-II receptor expressed from the cDNA, together with previously known features of the receptor, suggest that it is the same protein as this mannose-6-phosphate receptor.

Receptor purification and sequencing

The IGF-II receptor was purified from detergent-solubilized lysates of the Buffalo rat liver cell line BRL-3A by sequential affinity chromatography on wheat-germ-agglutinin-Sepharose and IGF-II Affigel (the IGF-II used throughout these studies was synthesized in *Escherichia coli* from the cDNA, and contains no sugar residues)²⁶. Because the BRL-3A cell line also expresses significant amounts of the IGF-I receptor (which binds the moderate affinity to the IGF-II Affigel), the IGF-I receptor was first removed from the lysates by passage over an affinity column composed of several monoclonal antibodies to the IGF-I receptor²⁷. By these procedures, 100 roller bottles of confluent cells yielded ~100–200 µg of IGF-II receptor protein (M_r ~250 K), which was homogeneous in SDS gel electrophoresis. Several lines of evidence suggest that the major component of this preparation is the IGF-II receptor: the protein binds IGF-II Affigel with high affinity; the purified 250 K protein is specifically labelled by covalently cross-linked ¹²⁵I-labelled IGF-II (not shown), and polyclonal antisera raised against these preparations inhibit the binding of IGF-II to its receptor on HepG2 cells²⁶. Receptor protein was applied to preparative SDS polyacrylamide gels, and the M_r 250 K region was selectively eluted, precipitated, and digested with trypsin²⁸. The resulting tryptic peptides were fractionated by reverse-phase HPLC, and 14 peptides were sequenced by automated Edman degradation on a gas-phase sequencer²⁸. The yields of amino acids from the peptides in these studies (not shown) indicate that the sequences obtained are all from the major component of the receptor preparation and not from a minor contaminant.

Isolation of receptor cDNA clones

The amino-acid sequence of one of the tryptic peptides (peptide 6: FLHQDIDSA in single-letter code) was used to design a 26-base oligonucleotide probe: TTCCTICATCAGGACATT-GACTCIGC (where I represents inosine²⁹). This synthetic oligonucleotide was used to screen a rat kidney λgt11 cDNA library, and two clones which hybridized strongly to the probe were chosen for nucleotide sequence analysis. The cDNA insert from one clone (K3, 2.4 kb) contained an open reading frame which included the amino-acid sequence of peptide 6. The cDNA insert of clone K3 was then used to screen a λgt10 cDNA library prepared from the human hepatoma cell line HepG2. Ten positive clones were obtained, all of which represented fragments of the same cDNA. One of these (H5.1, 4.5 kb insert) was used to rescreen the same library. Of the 24 clones obtained in the second screening, two clones (H8.3, 3.8 kb; H5.2, 4.3 kb) overlapped with H5.1 to provide a total of 8.6 kb of cDNA. Nucleotide sequencing of these cDNAs indicated that additional 5' sequences were lacking, and so an additional screen of the library was performed with a 150-base-pair (bp) *Pst*I-*Eco*RI fragment from the 5' terminus of H8.3. Six additional clones were isolated, and one of these (H5B, 1.7 kb) provided the extreme 5' sequences and brought the total length of the receptor cDNA to ~9.1 kb.

The size of the IGF-II receptor messenger RNA was confirmed by Northern blot hybridization with labelled rat and human receptor cDNA fragments (Fig. 1). IGF-II receptor cDNA probes hybridized to a single band in RNA isolated from various rat and human cell lines and tissues. The size of this band

(~9.0 kb) suggests that the cDNA isolated in these studies (9.1 kb) represents the full-length receptor mRNA. The amount of IGF-II receptor mRNA varied greatly among the cells and tissues tested. Adult tissues, including rat liver and kidney (Fig. 1) and several other tissues (human placenta, rat brain, lung, muscle: not shown) contain small amounts of IGF-II receptor mRNA. In contrast, IGF-II receptor mRNA is very abundant in several established cell lines (Fig. 1).

Primary structure of IGF-II receptor

The complete cDNA sequence contained in the four overlapping clones is 9,090 bp in length (Fig. 2). The cDNA includes a 147-bp 5' non-coding region; a single large open reading frame of 7,473 bp (nucleotides 148–7,620); and a 1,475-bp 3' non-coding region which includes a polyadenylation signal (AATAAAA) 11 bp upstream of a 3' poly(A) tract. The open reading frame encodes a protein of 2,491 amino acids, with a predicted M_r of 274,353. The amino-acid sequence contains all 14 of the tryptic peptides isolated and sequenced from the original rat IGF-II receptor preparation. The protein sequence begins with a 40-residue segment having the characteristic features of a cleavable signal sequence, including: a positively charged N-terminal region (22 residues), a hydrophobic central region (12 residues), and a small (six residues) polar C-terminal region. Recently developed methods³⁰ predict that the most probable site of signal peptide cleavage in the IGF-II receptor is after the alanine shown in Fig. 2 as residue -1. This prediction is supported by the N-terminal amino-acid sequence of the purified protein (not shown). Although we have demonstrated several overlapping N-terminal sequences (suggesting that the cleavage site varies), three major peptide components could be discerned which began at Gln 1, Ala 2 and Ala 3. Beyond the signal sequence, hydrophobicity analysis³¹ shows only one major hydrophobic segment (23 residues, positions 2,265–2,287) that is probably the transmembrane domain of the receptor; it is immediately followed by a dibasic sequence (Lys-Lys). It seems from these considerations that the IGF-II receptor is oriented almost entirely (92%) to the outside of the cell (2,264 N-terminal extracellular residues and 164 C-terminal cytoplasmic residues).

The receptor contains 19 potential extracellular N-linked glycosylation sites (Asn-X-Ser or Asn-X-Thr, where X is any amino acid). From the cDNA an M_r of 270,294 (after removal of the signal peptide) is predicted, and glycosylation is known to contribute 20 K–30 K to the receptor¹⁷; thus the M_r for the mature glycosylated receptor is ~300 K. This is somewhat greater than estimates of receptor size from SDS gel electrophoresis (M_r 250 K–270 K) (refs 13–17). Although post-translational modifications of the receptor could account for this discrepancy, it is more likely that the electrophoretic methods used to estimate molecular size are inaccurate, particularly for such large proteins.

The large extracellular domain of the IGF-II receptor is almost entirely made up of 15 conserved repeat sequences of ~150 residues, which are aligned in Fig. 3. Although the repeats are only 20% identical, they contain a highly conserved pattern of eight cysteine residues and several conserved hydrophobic regions near the cysteines and C terminus (see consensus sequence, Fig. 3a). Remarkably, very little of the external domain of the receptor is not encompassed by these structures: the first repeat begins soon after the signal sequence, and each repeat unit thereafter leads directly into the next, except for a small gap (20–30 residues) after repeat 14 and another small gap (15 residues) between repeat 15 and the transmembrane domain. The repeats end abruptly at the transmembrane domain. The only major interruption in the repeat sequences is an insertion of a 43-amino-acid segment after Cys 4 in repeat 13. This insertion is homologous to the type-II region of fibronectin, a disulphide-linked structure that is repeated in tandem fashion in the collagen-binding domain of fibronectin³² and is also found once in factor XII (ref. 33) and again in bovine seminal

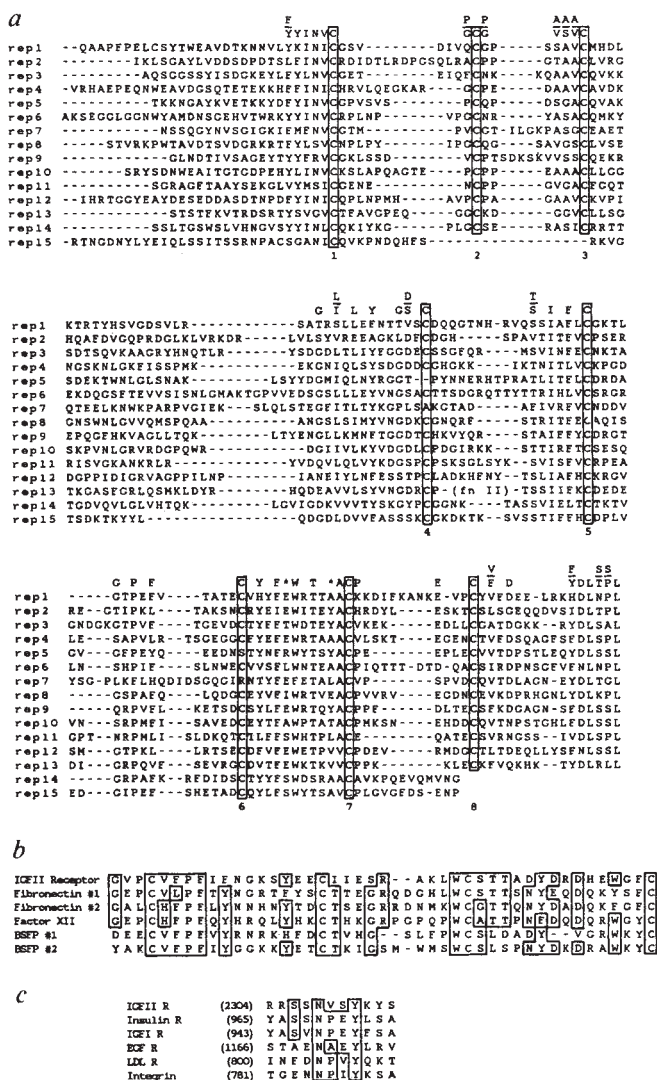


Fig. 3 *a*, Alignment of the fifteen repeat sequences in the external domain of the IGF-II receptor. Repeat 1 begins at residue 1 from Fig. 2*a*, and all repeats lead directly into the next, except for a 27-residue gap between repeats 14 and 15. Repeat 15 ends arbitrarily at position 2,249, 15 residues from the transmembrane domain. Gaps (dashed lines) have been introduced in the sequences to optimize alignments. Residues occurring eight or more times in a position are indicated in the consensus sequence above the repeat sequences; asterisks, positions where differences between residues are conservative. Conserved cysteines are boxed and numbered. The (fn II) notation indicates the insertion in repeat 13. *b*, Alignment of the insertion in repeat 13 (residues 1,860–1,902) with the type-II homology region found twice in fibronectin³², once in factor XII (ref. 33), and twice in bovine seminal fluid protein CDC-109 (BSFP) (ref. 34). Residues conserved in four or more sequences are boxed. *c*, Alignment of residues surrounding Tyr 2,311 of the IGF-II receptor with residues surrounding homologous tyrosines in other receptors (see text). Residues occurring at the same position in three of the receptors are boxed.

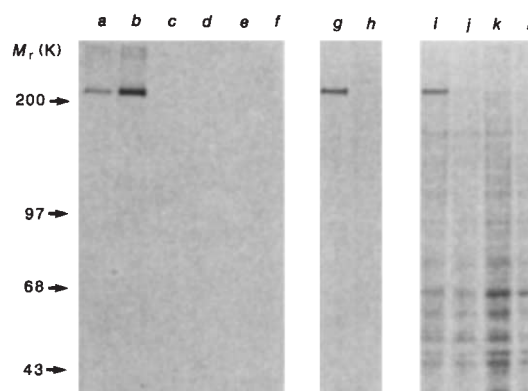


Fig. 4 Expression of the IGF-II receptor in *Xenopus* oocytes. A cDNA encompassing the 7.5-K coding domain of the IGF-II receptor was constructed from fragments of three overlapping cDNA clones (H5B, H8.3, H5.1), using convenient restriction sites (*Fsp*I, *Pf*MI, see Fig. 2*b*). The resulting cDNA fragment (7.7 kb) was placed in the BlueScribe vector (Stratagene) under the transcriptional control of the promoter for T3 RNA polymerase. Transcription *in vitro* of the linearized vector (using T3 polymerase according to Stratagene procedures) generated synthetic mRNA (7.7 kb) which was injected into *Xenopus laevis* oocytes⁵⁷. Oocytes were metabolically labelled for 16 h at 19°C with [³⁵S]cysteine, solubilized, immunoprecipitated, and analysed on 7% polyacrylamide gels as described⁵⁷. To decrease background, all precipitations (except those in lanes *i-l*) were done in the presence of 0.2% SDS. Each lane represents the equivalent of ~2 oocytes. Lanes *a-f* indicate immunoprecipitations of lysates from injected (*a-c*) and uninjected control (*d-f*) oocytes with rabbit antisera against the purified IGF-II receptor (*a, d*), the purified CIM6P receptor (S. Kornfeld) (*b, e*), or control serum (*c, f*). Differences in the amount of receptor precipitated by the two antisera reflect differences in their relative avidities for the receptor. Lanes *g* and *h*, precipitation of lysates from injected oocytes with 20 µl IGF-II Affigel (*g*) or Affigel coupled to rabbit immunoglobulins (used as a control ligand) (*h*). Lanes *i-l*, precipitation of lysates from injected (*i, j*) or uninjected (*k, l*) oocytes with IGF-II Affigel in the absence (*i, k*) or presence (*j, l*) of 1 µM IGF-II. The positions of pre-stained relative molecular mass markers (in thousands) are indicated.

Homology with mannose-6-phosphate receptor

The primary sequence of the human IGF-II receptor is strikingly similar to the recently characterized sequence of the bovine cation-independent mannose-6-phosphate (CIM6P) receptor, a protein implicated in the targeting of lysosomal enzymes to the lysosomes²⁵. The published partial sequence of the bovine CIM6P receptor cDNA (ref. 24) predicts the C-terminal 1,461 amino acids. These are 80% identical to the corresponding residues of the human IGF-II receptor, and include the same small hydrophilic cytoplasmic domain, a fibronectin type-II homology structure, and a large extracellular region made up of repeat sequences similar to those described above. The remarkable similarities between the two receptors suggest that

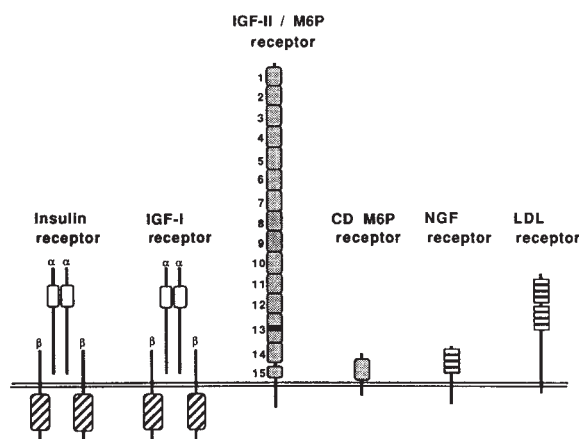


Fig. 5 Schematic comparison of IGF-II receptor structure with related receptors. All proteins are drawn to the same scale, and oriented in the plasma membrane with carboxyl termini inside. Structures are based on published primary sequences^{8-10,24,37,41-43}. Extracellular cysteine-rich regions and repeat sequences are indicated by boxes; these are shaded in the IGF-II and M6P receptors and numbered according to Fig. 3a. The dark band in repeat 13 represents the type-II fibronectin homology region. The tyrosine kinase domains of the insulin and IGF-I receptors are indicated by boxes containing diagonal lines.

they represent the human and bovine equivalents of the same protein. Identity of 80% between bovine and human sequences of a protein is normal for a protein evolving at a moderate rate⁴⁰. The partial sequence of the rat IGF-II receptor tends to support this conclusion. The evolutionary divergence of rats and humans occurred at the same time or soon after the divergence of cows and humans, and therefore the percentage of identical residues between rat and human sequences should be similar to that between cow and human sequences. Our partial sequence of the rat IGF-II receptor (about 300 extracellular residues, not shown) is 80–85% identical to the corresponding human sequence. Furthermore, the partial sequences of the rat IGF-II receptor and bovine CIM6P receptor are about 80% identical.

The sequence of the 46 K cation-dependent (CD) M6P receptor⁴¹, a second protein implicated in lysosomal enzyme targeting, reveals that its extracellular domain is made up of a 165-residue sequence that closely resembles the repeat units of the larger IGF-II/CIM6P receptor. There is no fibronectin homology region in the CDM6P receptor, and its cytoplasmic domain is unlike that of the larger receptor.

IGF-II receptor expression in *Xenopus* oocytes

To understand further the properties of the IGF-II receptor and its relationship to the CIM6P receptor, the protein was expressed in a heterologous cell system. Synthetic receptor mRNA, produced by transcription of the cDNA *in vitro*, was injected into *Xenopus* oocytes. Metabolically labelled lysates from these oocytes contain a large protein ($M_r > 250$ K) that is immunoprecipitated by antisera against the purified IGF-II receptor and also by antisera against the purified CIM6P receptor (Fig. 4, lanes *a–f*). The expressed protein from injected oocytes binds with high affinity to IGF-II covalently linked to an agarose support (IGF-II Affigel). This interaction with IGF-II is specific, because the protein does not bind to Affigel linked to an unrelated ligand (lanes *g* and *h*), and the binding of the receptor to IGF-II Affigel is inhibited in the presence of 1 μ M IGF-II (lanes *i–l*).

Discussion

The IGF-II receptor displays several structural features which distinguish it from other growth factor receptors (Fig. 5). These include a striking extracellular domain made up almost exclusively of 15 cysteine-based repeats, which show no primary structural relationship to the cysteine-rich regions found in the receptors for insulin^{8,9}, IGF-I (ref. 10), LDL (ref. 37), and nerve growth factor^{42,43}. But in all of its general structural features, and in 80% of its amino-acid residues, the human IGF-II receptor is identical to the bovine CIM6P receptor²⁴. This unexpected relationship between the two receptors is less surprising in that there are remarkable parallels in many of the previously

described properties of the two molecules. It is known that both receptors: are found predominantly inside the cell^{19,25,44}; are expressed at high levels in many cell types^{24,25} (Fig. 1); recycle between the plasma membrane and the cell interior^{25,46,47}; and display decreased electrophoretic mobility on SDS-polyacrylamide gels after reduction^{13-16,44}. Finally, as mentioned above, the minor difference between the bovine and human sequences of the two receptors is reasonably explained by evolutionary divergence. This conclusion is further supported by studies of IGF-II receptor expression in oocytes (Fig. 4). The protein encoded by the cDNA, which bound IGF-II with high affinity, was specifically immunoprecipitated by antibodies against both the IGF-II receptor and the CIM6P receptor. The simplest interpretation of these results is that the two binding activities reside in the same molecule.

The location of these distinct ligand binding activities within the extracellular domain of the receptor presents an intriguing puzzle. The number of mannose-6-phosphate (M6P) binding sites on the CIM6P receptor is difficult to evaluate because a single lysosomal enzyme (the presumed physiological ligand for this receptor) contains several phosphomannosyl residues. As a result, the binding of lysosomal enzymes to the receptor occurs with much higher avidity than the binding of single M6P molecules²⁵. The 150-residue repeat domain of the receptor is probably involved in M6P binding, as the extracellular domain of the 46 kD CDM6P receptor, which is composed of one such repeat unit, binds phosphomannosyl residues⁴¹. The CDM6P receptor seems to be an oligomer, and thus the high avidity binding of lysosomal enzymes might require interactions between two or more receptors⁴¹. Similarly, the multiple repeats of the IGF-II/CIM6P receptor may form a series of binding sites which interact to give high-avidity binding.

The structural basis of the IGF-II binding site is unclear, although Scatchard analysis of IGF-II binding¹⁹ supports the existence of a single site. The repeat units could participate in IGF-II binding, but it seems more likely that they are mainly involved in M6P binding. The two ligands do not appear to recognize the same site, as M6P does not inhibit IGF-II binding (R. Roth *et al.*, unpublished observations). This focuses attention on the fibronectin homology region, the only other extracellular domain. The type-II structures of fibronectin, factor XII, and protein CDC-109 probably provide the structural framework for binding sites whose specificities are defined by unique residues within, or adjacent to, the type-II region⁴⁸. For example, a unique 14-residue segment adjacent to the type-II structure of fibronectin is crucial for its collagen-binding activity⁴⁹. Thus, the type-II structure of the IGF-II receptor, combined with certain adjacent residues, could contribute to an IGF-II binding site.

The lack of sequence homology suggests that the structure of the IGF-II receptor binding site bears no relationship to those

of the insulin and IGF-I receptors. This may explain the differences in ligand specificity between the receptor types. Unlike the insulin and IGF-I receptors, the IGF-II receptor does not bind insulin^{7,11,12} and thus might recognize a unique region of the IGF molecule. The dramatic differences between the two classes of IGF receptor suggest distinct evolutionary origins and support the existence of at least two distinct families of IGF binding proteins. The serum IGF carrier proteins, which display ligand specificities similar to that of the IGF-II receptor (that is, no affinity for insulin)^{50,51}, might therefore be related to the IGF-II receptor class of binding proteins. Although a thorough comparison of the IGF-II receptor with IGF carrier proteins awaits more complete sequence information, published N-terminal sequences of the major IGF carrier proteins secreted by BRL-3A cells⁵⁰ and HepG2 cells⁵¹ (M_r 36 K) are not contained in the IGF-II receptor sequence. A high molecular weight IGF binding protein has, however, recently been identified in fetal rat serum⁵². The ligand binding specificity and antigenic structure of this protein are similar to those of the IGF-II receptor, and its size is slightly less than that of the membrane-associated receptor⁵³. Thus, proteolytic cleavage could release a truncated receptor, which might then act as a soluble IGF-binding protein. Although a single mRNA was detected by Northern analysis (Fig. 1), it is also possible that alternative splicing of the receptor mRNA results in a truncated receptor. In any case, a serum transport protein and transmembrane receptor molecule may originate from the same gene.

The mechanism of action of the membrane-bound form of the IGF-II receptor is not yet understood. The interaction of IGF-II with the IGF-I or insulin receptors can account for many IGF-II actions^{7,12,21-23}, but certain IGF-II effects are probably mediated by the IGF-II receptor²⁶. Thus, the structure of the

intracellular domain and its possible function in transmembrane signalling are of great interest. Homology searches failed to detect a relationship between this domain and any known coupling or effector molecules involved in signal transmission, but the IGF-II receptor could act together with another receptor or signalling molecule. This seems more likely than a mechanism involving a catalytic activity located within the receptor itself.

These considerations of the multiple potential functions of the IGF-II receptor are interesting in view of our findings, suggesting that this receptor is identical, to the CIM6P receptor. The interaction of a lysosomal targeting protein with a growth factor raises several questions concerning the integration of diverse functional activities in one receptor. Does the receptor transport IGF-II into the cell, where the ligand fulfills some intracellular function? Is the activity, turnover, or transport of lysosomal enzymes coupled to the actions or endocytosis of IGF-II? Do the M6P binding sites of the receptor interact with other ligands involved in intercellular communication or growth regulation? The further development of systems for expression of normal and mutated receptor cDNAs will make it possible to approach these questions and identify the domains of the receptor which contribute to its apparently multiple functions.

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Can Planck-mass relics of evaporating black holes close the Universe?

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The fate of an evaporating black hole when it reaches the Planck mass is a matter for conjecture. Here, we propose that the cosmological dark matter consists of the Planck-mass remnants of evaporating primordial black holes. Such remnants would be expected to have close to the critical density if the black holes evaporating at the present epoch have the maximum density consistent with cosmic-ray constraints. The remnants are also candidates for the missing mass in the galactic halo. Primordial black holes of the required density may form naturally at the end of an inflationary epoch. Planck-mass relics would behave dynamically just like 'cold dark matter' and would therefore share the attractions of other 'cold' candidates. In addition, because the baryonic matter in black holes cannot participate in nucleosynthesis the limits on the baryonic content of the Universe set by primordial nucleosynthesis are circumvented.

The Hawking derivation¹ for the quantum thermal emission of particles from black holes breaks down when the black-hole mass is close to the Planck mass, $M_{\text{pl}} = [\hbar c / G]^{1/2} = 2.18 \times 10^{-5}$ g, because the size of such a hole is comparable with the Compton wavelength associated with its mass; also the timescale of the mass loss is comparable with the frequency of the quantum radiation². Indeed, extrapolating semiclassical back reaction³ and surface correction⁴ terms to this régime implies that the emission process stops. All semiclassical approaches, however, break down at M_{pl} . Three possibilities for what happens next are: (1) a stable object⁵ forms with a mass $\sim M_{\text{pl}}$; (2) a naked space-time singularity⁶ is left, or a singularity in which all information is lost⁷ or (3) a terminating explosion occurs, in which the singularity vanishes⁸. We note that (1) says nothing about whether the final state, which may have a residue mass, vanishes. Suggestion (3) is based on the use of the CPT theorem to show that⁹ if a volume initially contained no singularity then it must return to a state with no singularity, but it assumed that the time-reversal of a black hole is a black hole, or equivalently, that a black hole and a white hole are indistinguishable. The assumption, and the entropy argument also given in ref. 9, remain controversial^{5,10}. Here we explore the consequences of (1) and (2).

Previous authors have shown¹¹ that it may have been possible for black holes to have formed in the early Universe. If the holes formed from initial density perturbations at time t , their formation mass would be

$$M_i = \gamma^{3/2} M_H(t) \quad (1)$$

where the equation of state is $p = \gamma\rho$ and M_H is the horizon mass. For a Friedmann model with zero curvature,

$$M_H = c^3 t / G = 4.04 \times 10^{38} \frac{t}{\text{sec}} \text{ g} \quad (2)$$

For scale-invariant perturbations, the number density of holes with initial mass in the range $(M, M + dM)$ would be¹²

$$dn = (\alpha - 2) \Omega_{\text{pbh}} \rho_{\text{crit}} M_*^{\alpha-2} M^{-\alpha} dM, \quad (3)$$

$$\alpha = (1 + 3\gamma) / (1 + \gamma) + 1$$

where $0 < \gamma < 1$ and Ω_{pbh} is the fraction of the present critical density ρ_{crit} in holes larger than M_* , the mass of a black hole that would have just evaporated by today. During most of the history of the early Universe, one expects the equation of state

to be radiation-dominated, so $\gamma = 1/3$ and $\alpha = 5/2$. From (3), equation (3) implies that the present number density of Planck-mass relics left by the evaporation of primordial holes is

$$n_{\text{pl}} = \int_{M_{\text{min}}}^{M_*} \frac{dn}{dM} dM = \frac{1}{3} \Omega_{\text{pbh}} \rho_{\text{crit}} M_*^{1/2} M_{\text{min}}^{-3/2} \quad (4)$$

where M_{min} is the mass of the first holes that formed. Also, if Ω_{pl} is the density of the Planck mass objects in units of the critical density, we must have

$$n_{\text{pl}} = \Omega_{\text{pl}} \rho_{\text{crit}} / M_{\text{pl}} = 8.63 \times 10^{-25} \Omega_{\text{pl}} h^2 \text{ cm}^{-3} \quad (5)$$

for a Hubble constant $H_0 = 100 h \text{ km s}^{-1} \text{ Mpc}^{-1}$. Therefore

$$\Omega_{\text{pbh}} = 3 \Omega_{\text{pl}} (M_{\text{min}} / M_*)^{1/2} (M_{\text{min}} / M_{\text{pl}}) \quad (6)$$

For a flat Friedmann model, M_* ranges (J. H. MacGibbon, in preparation) from 4.02×10^{14} g if $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ to 5.27×10^{14} g if $H_0 = 40 \text{ km s}^{-1} \text{ Mpc}^{-1}$. In this range $M_* = 4.02 \times 10^{14} h^{-0.3}$ g which gives

$$\Omega_{\text{pbh}} = 7.0 \times 10^{-10} \Omega_{\text{pl}} h^{0.15} (M_{\text{min}} / M_{\text{pl}})^{3/2} \quad (7)$$

(Lowering the total cosmological density will raise M_* only slightly.)

The most unambiguous limits on Ω_{pbh} come from comparing the photon emission of primordial black holes evaporating today with the observed γ -ray background radiation in the 100 MeV–1 GeV range. Recent calculations (J. H. MacGibbon, in preparation), allowing for the evaporation of quark and gluon jets, place a γ -ray background limit on Ω_{pbh} of

$$\Omega_{\text{pbh}} \leq 7.9 (\pm 3.0) \times 10^{-9} h^{-2.0 (\pm 1.0)} \quad (8)$$

Thus in equation (7), we require

$$M_{\text{min}} / M_{\text{pl}} \leq 5.0 (\pm 1.8) \Omega_{\text{pl}}^{-2/3} h^{-1.4 (\pm 0.7)} \quad (9)$$

The comparisons with the observed antiproton and positron spectra also give similar constraints but are dependent on uncertain factors such as the degree to which the holes are clustered inside our galactic halo and the time for the particles to leak out of the halo. Indeed, it has been suggested that the antiproton and positron spectra could be explained by black-hole evaporation (J. H. MacGibbon, in preparation), in which case one interprets equation (8) as an equality.

In a Friedmann universe without inflation, we expect the equation of state to be radiation-dominated all the way back to the Planck time and so $M_{\text{min}} \approx 0.2 M_{\text{pl}}$. Because it does not make sense to discuss black holes with mass below M_{pl} , we take $M_{\text{min}} = M_{\text{pl}}$. Condition (9) is satisfied then for all $h < 1$ and $\Omega_{\text{pl}} \approx 0.1 - 1$. The Planck-mass relics can comprise a significant amount of the missing matter in the Universe, including even $\Omega_{\text{pl}} \approx 1$. They are also candidates for the missing matter in the galactic halo, $\Omega_h \sim 0.1$.

In the inflationary model¹³⁻¹⁵ only black holes produced from density fluctuations after the time of inflation contribute to the initial mass spectrum. Those formed before inflation or during its early stages are diluted to a negligible density by the exponential increase in the cosmic scale factor. Thus the appropriate value for M_{min} is given by the horizon mass after reheating. For a reheating temperature T_R and the Planck temperature T_{pl} , equation (1) implies

$$M_{\text{min}} = 4.2 \times 10^{-6} (T_R / T_{\text{pl}})^{-2} \text{ g} \quad (10)$$

To satisfy equation (9), we require

$$T_R \geq 2.0 (\pm 0.7) \times 10^{-1} h^{0.7 (\pm 0.4)} \Omega_{\text{pl}}^{1/3} T_{\text{pl}} \quad (11)$$

The effect of gravitons produced during inflation on the anisotropy of the microwave background¹⁶ places an upper limit on the reheating temperature of

$$T_R \leq 3.0 \times 10^{-3} \eta^{-1/4} T_{\text{pl}} \quad (12)$$

where $\eta \approx O(10^2)$ is the effective number of degrees of freedom

in the assumed grand unified theory. For $h \approx 0.3 - 1.0$, $\Omega_{\text{pl}} \approx 1$ does not seem possible in the inflationary model unless the graviton limit can be circumvented. This could occur if the large-scale components of the gravity-wave spectrum produced by the vacuum fluctuations in the expanding Universe were suppressed. A density $\Omega_{\text{pl}} \approx 0.1$ may be possible, however, for small h , when we allow for the statistical error in the limit (11).

We have shown that $\Omega_{\text{pl}} \approx 1$ is compatible with the constraints on Ω_{pbh} at least for a Friedmann universe. Indeed, if the positron or antiproton spectra are to be explained by primordial black-hole emission, we expect $\Omega_{\text{pl}} \approx 1$. This requires that the r.m.s. value for gaussian initial density perturbations to be $\delta_{\text{r.m.s.}} \leq 0.03$ on mass scales $< M_*$. This is somewhat larger than the cosmic microwave background anisotropy limits¹⁷ on $\delta_{\text{r.m.s.}}$ on much greater scales, but models^{18,19} have been proposed in which the perturbations on small scales may become decoupled from those on large scales. Although it may seem *a priori* unlikely that $\delta_{\text{r.m.s.}}$ would have the value required to ensure $\Omega_{\text{pl}} \approx 1$, we note that the relics are bound to have the critical density in the inflationary or phase transition scenario if the fraction of the Universe going into the initial holes is large enough that they dominate the radiation density today. This happens provided at the formation redshift z_f , the ratio of the density in black holes to the radiation density is $\geq 10^{-3}(1+z_f)^{-1}$.

We have argued that Planck-mass relics could provide $\Omega_{\text{pl}} \approx 1$. They are also candidates for the missing mass in the halo. Because most of the remnants will have come from the holes with the smallest initial masses, they need not have the same distribution as those evaporating today. The remnants behave like cold dark matter and hence one could invoke biased galaxy formation²⁰, in which the relics explain both the halo and the

background dark matter. We also note that black holes could form at various phase transitions in the early Universe. For these, equation (3) does not apply and their spectrum peaks near the horizon mass at the time of the phase transition. Although it does not seem as natural as our scenario, sufficient numbers of holes may have formed for the density in the Planck mass relics today to close the Universe. This may also occur for black holes forming from initial-density perturbations even if $\delta_{\text{r.m.s.}}$ is not constant on all scales $< M_*$.

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Formation of a millisecond pulsar in a globular cluster

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The discovery of an isolated 3-millisecond pulsar PSR1821-24 in the globular cluster M28 (=NGC6626)^{1,2} has important implications for the genesis of such objects. Millisecond pulsars (MSPs) have weak ($\sim 10^9$ G) magnetic fields and are commonly believed to be spun up during an epoch of mass transfer in a close binary system. However, the modest cluster escape velocity, $v_{\text{esc}} \leq 40 \text{ km s}^{-1}$, implies that the pulsar must be extracted from the binary with some care. One possibility, often discussed in connection with the formation of the isolated MSP 1937+214, involves coalescence with the mass-donor companion (ref. 3, and refs therein). In this letter, we suggest a model appropriate to the cluster site, namely disruption of an expanded tidal capture binary by the perturbations of field stars. We also attempt to relate the MSP formation rate to that of the cluster X-ray sources.

The simplest model for PSR1821-24's formation would have it born with its present high spin rate and low field. However, as no massive star formation has occurred in the past $\tau_{\text{cl}} \sim 10^{10}$ yr, this would require both a remarkably cold, placid birth and an unprecedented spin-down age of order the Hubble time. In contrast, the standard interpretation has all pulsars born with modest spin rates and fields of order 10^{12} G. After their initial field has decayed to $\leq 10^9$ G in $\leq 10^8$ yr, MSPs accrete $\sim 0.1 M_{\odot}$ from a companion and are spun up to their present periods⁴.

In a globular cluster, the initial massive star evolution should

produce $\geq 3 \times 10^3$ neutron stars. The binary-induced velocities of galactic pulsars ($\sim 100 \text{ km s}^{-1}$) and the mass-to-light limits on cluster cores (I. King, personal communication; ref. 5) suggest that some modest fraction, ≤ 0.1 , of these neutron stars avoid escape, leaving $\sim 300 N_{300}$ neutron stars (where N_{300} is the number of neutron stars divided by 300) in the cluster core. These neutron stars can form tight binaries through dissipative two-body tidal captures⁶ of cluster main sequence dwarfs on a timescale $\tau_{\text{ic}} \sim (6\pi n R_3 G(m_1 + m_2)/v)^{-1} \sim 10^{11} (n_{i,5} R_i m_i v_6)^{-1} \text{ yr}$ where $n_{i,5}$, R_i , and m_i are the density (in units of 10^5 per pc^3), radius and mass (in solar units) for stars of type i , and v_6 is the velocity dispersion of the cluster in units of 10 km s^{-1} . Thus, roughly $\tau_{\text{cl}}/\tau_{\text{ic}} \sim 0.1$ of the neutron stars experience such encounters in a Hubble time. More detailed computations^{7,8} confirm this result and show that although two thirds of the encounters result in circular orbits with separation $a \sim 6 R_*$, about a third are direct impacts leading to coalescence. With the above estimates $\sim 20 N_{300}$ neutron stars are captured into binaries.

The stellar mean mass, $\bar{m} \sim M_{\odot}$ and velocity v in the cluster core define an energy, $kT \sim \bar{m}v^2$. As the gravitational disruption probability of a binary with a binding energy $kT = 2.9$ is 50% (ref. 9), a tidal capture system whose subsequent evolution expands the orbit substantially can become unbound by close encounters with field stars. Such expansion seems feasible only for captures of cluster giants and subgiants. From evolutionary models¹⁰ we can estimate that a star of $0.8 M_{\odot}$ and metallicity $Z = 10^{-4}$ will spend $\sim 3 \times 10^8$ yr with $R_* > 3 R_{\odot}$ (hereafter 'sub-giant') and $\sim 10^8$ yr with $R_* > 7 R_{\odot}$ (hereafter 'giant'). Following Verbunt¹¹, we use the linear dependence of τ_{ic} on R_* , and weight by the number of stars at each stage, finding that the 'giant' and 'subgiant' groups each capture $\sim 1 N_{300}$ neutron star in τ_{cl} .

At this early stage of the giant evolution the core mass should be $\leq 0.1 M_{\odot}$; nevertheless, the modest inflation of the envelope and the effect of tidal heating (A. Ray, personal communication) suggest that somewhat greater than 1/3 of the tidal captures will lead to coalescence. Estimating the orbital capture fraction at 0.5, we consider the fate of the 'giant' encounters, for which

majority of known pulsars (98.5%) being solitary objects.

In summary, a simple direct way of making millisecond pulsars with low magnetic fields is to form them from contact binary white dwarfs systems of sufficient total mass to form a neutron star. Whether or not the system remains binary may depend on finer details. The idea of recycling old pulsars (which served the important role of calling attention to pulsars in old stellar populations) depends on rapid magnetic field decay, a phenomenon which may not even exist.

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Formation of isolated millisecond pulsars in globular clusters

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After previous searches for radio pulsars in globular clusters^{1,2}, a radio pulsar with a period of 3.054 millisecond has now been discovered in NGC6626 (M28)³. The upper limit of 3×10^{-17} to the observed period derivative indicates that the pulsar is not in a binary³. Millisecond pulsars are probably 'recycled' old pulsars which have been spun-up through accretion from a binary stellar companion^{4,5}. In a globular cluster such a binary is likely to have formed in a tidally dissipative collision between an old neutron star and a low-mass field star from the cluster. Eventually, a mass-transfer phase ensues and the accretion of only $\sim 0.1 M_{\odot}$ from the companion is sufficient to spin up the neutron star to millisecond periods. Later in the history of the binary, it can undergo a catastrophic collision with a passing field star from the cluster, the net result of which may be a neutron star with a massive disk orbiting it. After much of the material is expelled via accretion-generated radiation pressure, only an isolated millisecond radio pulsar remains. We also explore the possibility that accretion from a massive disk is able directly to spin up the neutron star; in this case, however, the predicted number of millisecond pulsars in globular clusters would be ten times the number of bright low-mass X-ray binaries. In the model where the neutron star is spun up in a binary the predicted number of millisecond pulsars is significantly smaller. If the millisecond pulsar in NGC6626 has a magnetic moment similar to that of the other three known millisecond pulsars, the epoch of spin-up must have terminated $\leq 8 \times 10^8$ yr ago.

Millisecond pulsars and some low-mass X-ray binaries share a common evolutionary track^{4,5,25}, including an accretion spin-up phase. It is therefore reasonable that, because the formation of X-ray binaries in globular clusters is different from that in the plane⁶, the formation of millisecond pulsars in globular clusters probably follows different paths than their formation in the galactic disk. In the context of the accretion spin-up model, one can set constraints on the surface dipole magnetic field strength and age of the 3-ms pulsar. The shortest rotation period to which a neutron star with a surface dipole magnetic field strength B_0 (in units of 10^9 G) can be spun up for a given accretion rate $\dot{M}$ is the so-called equilibrium spin period P_{eq} given by⁷

$$P_{eq} \approx (1.9 \text{ ms}) B_0^{6/7} (\dot{M}/1.4 M_{\odot})^{-5/7} (\dot{M}/\dot{M}_{Edd})^{-3/7} R_6^{18/7} \quad (1)$$

where M and R_6 are the mass and radius of the neutron star in solar units and units of 10^6 cm respectively, and $\dot{M}_{Edd}$ is the maximum possible (Eddington limit) accretion rate of $\sim 1.5 \times 10^{-8} M_{\odot} \text{ yr}^{-1}$.

Equation (1) shows that the lower limit to the rotation period, for given neutron star parameters B , M and R_6 , occurs for $\dot{M} = \dot{M}_{Edd}$. For $M = 1.4 M_{\odot}$, $R_6 = 1$ this shortest period is

$$P_{min} = (1.9 \text{ ms}) B_0^{6/7} \quad (2)$$

This relation is indicated by the spin-up line in Fig. 1, in which all known binary and millisecond radio pulsars are indicated. Also indicated are the 'death line', beyond which radio pulsars stop pulsing⁸, and the 'Hubble' line, where pulsars originating on the spin-up line and spinning down by the emission of magnetic dipole radiation will be found after one Hubble time (1.5×10^{10} yr) under the artificial constraint that they evolve at constant surface dipole magnetic field strength.

Equation (2) yields an upper limit to the surface dipole magnetic field strength of the 3-ms pulsar of 1.7×10^9 G. (Taking the uncertainties in the mass and radius of the neutron star into account, this limit is $\sim 2.0 \times 10^9$ G.) For stronger fields it is unlikely that the pulsar will be spun up to 3 ms, even at the Eddington limit accretion rate.

Although there is evidence⁴ that when neutron stars are young their surface magnetic fields decay on a relatively short timescale ($\sim 10^7$ yr), the optical identification of the companions of three binary radio pulsars^{9,10}, as well as statistical arguments regarding the millisecond pulsars^{7,11} provide convincing evidence that the surface dipole field strengths of neutron stars do not decay below a value of $\sim 0.5 \times 10^9$ G. Interestingly, the observed $\dot{P}$ values of the three other millisecond pulsars all yield similar dipole surface magnetic field strengths of $(4.6 \pm 0.5) \times 10^8$ G. (Note however that the B -field of 0655+64, which has a pulse period of 0.196 s and an estimated age⁹ of 5×10^8 yr, is $\sim 10^{10}$ G.) For the moment we assume also that the surface dipole field strength of the millisecond pulsar in NGC6626 is not below 4.6×10^8 G.

Spin-up at the Eddington-limit accretion rate with this magnetic field strength leads to a shortest plausible spin period (on the spin-up line) of ~ 1.0 ms. The time required to spin down by emission of magnetic dipole radiation from this value to 3 ms, at $B_s = (4.6 \pm 0.5) \times 10^8$ G is $(6.3 \pm 1.2) \times 10^8$ yr (where the uncertainties are again due to the uncertainties in M and R_6). For any higher B -value the time required to spin down from the spin-up line to 3 ms is shorter. Therefore, we conclude that for a B -field of $(4-5) \times 10^8$ G the accretion phase of the 3 ms pulsar terminated $\leq 8 \times 10^8$ yr ago.

Single neutron stars in globular clusters may undergo close encounters with a variety of other cluster stars¹². The most common encounter is with a main-sequence star¹³. The cross-section for a close encounter is proportional to the distance of the closest approach, and capture ensues when the closest approach is less than ~ 3 times the radius of the main-sequence star. Thus one-third of the capture processes involve a direct

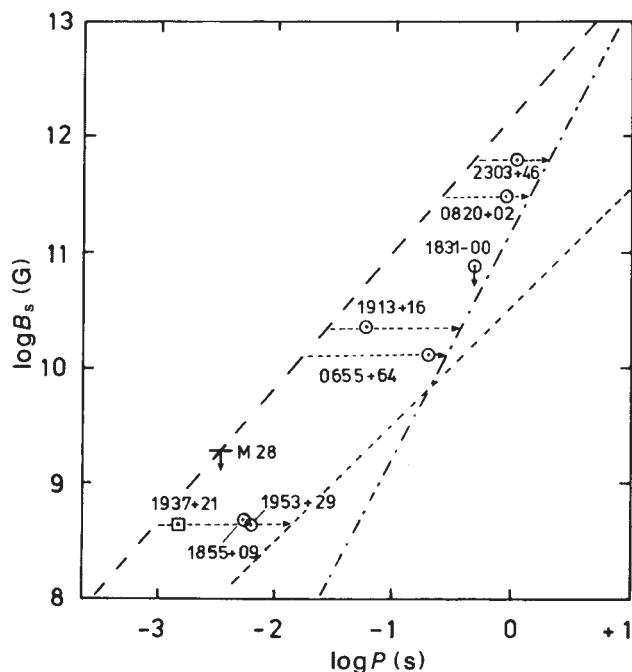


Fig. 1 B_s against P , showing the predicted upper limit to the magnetic field strength of the pulsar in M28 together with the positions of the seven binary pulsars and the 1.55-millisecond pulsar. Also shown are the 'spin-up' line (—), given by equation (2), the 'death line'⁸ (---), and the $\dot{P}$ line (....), the final period reached after a Hubble time if spin-down proceeds at constant B_s , along the horizontal arrows.

collision between the neutron star and the main-sequence star¹³. It has been proposed¹⁴ that such a direct hit leads to a complete disruption of the main-sequence star into a massive disk around the neutron star, and that the neutron star subsequently accretes from the disk at a rate of $\sim 10^{-9} M_{\odot} \text{ yr}^{-1}$. If this were the case, all such systems would evolve into millisecond pulsars; one-third of the globular cluster X-ray sources would become millisecond radio pulsars¹⁴. From the statistics of millisecond pulsars and low-mass X-ray binaries in the galactic disk, it can be shown that millisecond pulsars must have a lifetime 20–30 times that of low-mass X-ray binaries^{7,11}. Thus if one-third of all low-mass X-ray binaries in globular clusters evolve into millisecond radio pulsars, the predicted number of such pulsars in globular clusters is ~ 100 , ten times the number of low-mass X-ray binaries in globular clusters. Such numbers are close to present-day observational limits¹. If the millisecond pulsars lose rotational energy through magnetic dipole radiation, their number distribution will be strongly peaked at long periods. Inspection of the values of $S_{1,465} d^2$ (where $S_{1,465}$ is the flux density at 1,465 MHz and d the distance) for the known millisecond radio pulsars enables us to estimate the luminosity decrease (assuming the same constant value for B in all systems). We then find that only a small fraction of millisecond radio pulsars in globular clusters can be detected above a threshold of 1 mJy, amounting to 5% in all globular clusters, and $\sim 12\%$ in the sample observed by Hamilton *et al.*¹.

There is a problem for the massive disk formation model for the formation of globular cluster X-ray sources and millisecond radio pulsars. Accretion disks in cataclysmic variables¹⁵ with a mass of $\sim 10^{-9} M_{\odot}$ sustain a mass flow of $\sim 10^{-9} M_{\odot} \text{ yr}^{-1}$. It seems likely that a disk with a mass $M_d \sim 0.1 M_{\odot}$, i.e. about eight orders of magnitude more massive, has a mass flow $\dot{M}_{\odot}$ much in excess of the Eddington limit, in which case most of the mass is not accreted onto the neutron star, but expelled by radiation pressure. In this case the amount of accreted mass $(\dot{M}_{\text{edd}}/\dot{M})^{-1} \dot{M}_d < 0.1 M_{\odot}$ is not sufficient to lead to spin up to

millisecond periods, and other ways to form millisecond pulsars in globular clusters should be explored. We consider this next.

Two thirds of the close encounters between a neutron star and a main-sequence star lead to the formation of a close binary. In the course of ordinary binary evolution, the neutron star will accrete mass from its companion, and be spun up. But it can only show up as a radio pulsar after mass transfer from its companion stops completely. It is doubtful that it can do so in the course of ordinary evolution of a binary in which the mass transfer is driven by gravitational radiation or magnetic braking (and therefore not driven by the internal evolution of the main-sequence companion). In this case mass transfer will continue at an ever decreasing rate and the neutron star can never show up as a radio pulsar^{16–19}. In a globular cluster, however, there is a non-negligible chance that the binary may undergo a close encounter with a third star²⁰, which may cause the neutron star to become single. In a centrally dense cluster the expected number of collisions in the Hubble time is of the order unity for a compact binary²¹. The relative velocities in a globular cluster are too small for this third star to be able to 'ionize' a compact binary. The remaining possibilities are an exchange or a catastrophic encounter. In an exchange the incoming star takes the place of one of the binary stars and the least-massive star is more likely to be expelled. Because the neutron star is much more massive than its companion, the probability of its expulsion is small. In most close encounters a temporary triple star will be formed. The life time of the triple system is typically some hundred initial binary periods, and during this phase physical collisions between the stars are quite likely^{14,21,22}. Such collisions can lead to the destruction of the main-sequence stars, and to the formation of a massive disk around the neutron star, formed from the matter of one or even both of the two other stars. Again, it is likely that most of the mass of this disk is expelled during super-Eddington accretion, and that the small amount of matter settling on the neutron star does not affect its rotation much. But due to its spin up by accretion during its preceding binary phase, it may at this moment already be a millisecond pulsar. Once the disk has disappeared, the neutron star will show up as a radio pulsar, single or possibly in a binary. The formation rate through this process is less than the formation rate of low-mass X-ray binaries, as not all neutron stars in binaries are spun up to millisecond periods, and not all catastrophic encounters leave an isolated neutron star²¹. The lower formation rate of millisecond pulsars is compensated for by their longer life time, and from the binary spin-up model we estimate a total number between 10 and 100 of single millisecond pulsars formed in globular clusters over the last 15 billion years. Some of these may have escaped from the cluster due to recoil velocity following the triple star interaction¹⁴.

There is an other route to the same end result. Single neutron stars can undergo close encounters with giants^{12,23}. Direct hits may well lead to the formation of an ultra-short period binary through spiral-in of the neutron star in the giant atmosphere, whereas the other captures probably lead to binaries consisting of a giant and a neutron star²⁴. The neutron star/giant binaries will eventually evolve to moderately wide binaries consisting of a low-mass white dwarf and a millisecond pulsar^{25–30}. For initial separations of at most a few $R_{\odot}$, the final separation is ~ 6 times the original separation²⁵, too small to enable third stars in the cluster to ionize the binary, as these binaries are still 'hard'³¹. As the white dwarf is very far from filling its Roche lobe, and therefore does not transfer mass to the neutron star, this route leads to binary millisecond pulsars in globular clusters, which are similar to the millisecond binary pulsars PSR1855+09 and 1953+29. The formation rate of such binaries is $\sim 7\%$ of the formation rate of main-sequence star/neutron star binaries¹² which leads to a present number of millisecond pulsars in wide binaries with white dwarf companions approximately equal to the total number of low-mass X-ray binaries, ~ 10 .

Single millisecond pulsars may be formed from these binaries in a similar way as from neutron-star/main-sequence-star binaries, when a close encounter with a passing field star leads to the formation of a temporary triple star. A collision between the white dwarf and the main-sequence star can disrupt both stars and leave the neutron star with a massive disk. The cross-section of a binary to an encounter with a third star is proportional to the size a of the semi-major axis³¹. For the total number of catastrophic encounters with third stars the larger size of the white dwarf/neutron star binary just compensates its smaller formation rate, and therefore yields a number of single millisecond radio pulsars similar to that formed through catastrophic encounters of third stars with binaries consisting of a main-sequence star and a neutron star.

Finally, we mention that actual measurement of $\dot{P}$, and thereby

of B for the 3-ms radio pulsar in NGC6626 is interesting from the evolutionary point of view in two ways. In principle, millisecond pulsar with a low magnetic field strength might be the remnant of a young, rapidly rotating pulsar born with a low field strength. Due to the short lifetime of their massive progenitors, neutron stars in globular clusters must be almost as old as the clusters themselves. (More recent formation of neutron stars through white dwarf implosion is possible, but rare^{12,24}.) The above given limit on the age of the millisecond pulsar, obtained through equation (2), in fact excludes this possibility, unless the magnetic field strength B is much lower than our adopted lower limit of 4.6×10^8 G. Furthermore, in the binary spin-up model a measurement of B gives through equation (1) a lower limit on the $\dot{M}$ required to achieve a period as short as measured for the 3 millisecond pulsar in M28.

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Particle acceleration and production of energetic photons in SN1987A

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Young supernova remnants are likely to be bright sources of energetic photons and neutrinos through the collision of particles accelerated inside the remnant¹⁻³. Interactions of accelerated particles in the expanding envelope or in ambient radiation fields will also produce secondary photons and neutrinos at some level. If $>10^{39}$ erg s⁻¹ in protons above 10 TeV is injected into the target region, TeV photons from SN1987A could be observable with present detectors⁴⁻⁶. Synchrotron X rays and γ -rays up to 10 MeV, generated by accelerated electrons, may well also be detectable. We discuss a pulsar wind model for acceleration of particles and find that it would produce observable signals if the spin period of the pulsar is ≤ 10 ms.

Energetic photons will come from decay of neutral pions produced in collisions of protons in the material of the envelope, and by radiation of accelerated electrons and positrons. We have previously evaluated the related neutrino-induced, upward muon signal that would be produced from decay of charged pions and which can be searched for with large underground detectors in the Northern Hemisphere (ref. 7; H. Sato, Kyoto University). The expected spectrum of energetic photons is somewhat more model-dependent than that of the neutrinos, which can be produced only by nuclear interactions and by photopion production. Photons will also be attenuated to some extent on their way out of the production region. It is nevertheless likely that the photon signal will be more readily detectable than the neutrino signal because of the small cross-section of neutrino interactions at the detector.

To calculate the expected photon flux we need to know the spectrum of parent protons and electrons, the nature of the region in which they propagate after acceleration, and the magnetic field and radiation environment which determines the subsequent fate of produced photons. The signal depends primarily on the power in accelerated protons and electrons, and various models of acceleration may give observable signals. Table 1 shows the expected fluxes of photons at Earth above various threshold energies, for accelerated protons with power-law spectra of differential index γ . The normalization corresponds to 10^{40} erg s⁻¹ of protons with energies between 1 and 10^8 GeV injected into the nebula 50 kpc away. (In the pulsar wind model this power requires a period ≤ 8 ms for the central pulsar.) The numbers in Table 1 scale linearly with the power; in calculating them we have included only photons from decay of neutral pions produced by collisions of accelerated protons that have been injected into the nebula. We assume the protons have an infinite path length in the nebula and that the nebula is optically thin to the produced photons. The first assumption is valid provided the interaction rate of protons is large compared both to their rate of escape from the nebula and to its expansion rate. The second assumption becomes valid after the system has expanded for a few months. All secondary as well as primary interactions of injected protons are included, and all pions and kaons were assumed to decay before interacting. In this model, the accelerated protons will diffuse through the nebula, making occasional collisions that give rise to the photons that constitute the signal. One would not expect a signal with such a diffuse origin to be pulsed, so the threshold for detection will be relatively high. For example, a d.c. signal less than a few times 10^{-11} cm⁻² s⁻¹ would be very difficult to detect with an air Cherenkov detector (K. E. Turver, personal communication).

If protons are accelerated to energies $>10^7$ GeV, PeV photons capable of triggering surface-air-shower arrays will also be produced, although observation of these photons will be difficult because of the degradation of the flux in collisions with the 3-K background radiation in the 50-kpc distance to the Large Magellanic Cloud⁸. Protheroe⁹ has shown that the flux can be significantly regenerated by electron inverse Compton scattering

on the background radiation if the average intervening field is $<10^{-7}$ G. The cascading will produce a pile-up of photons below the absorption feature. Because of the uncertainty in the field, the extent of this effect is uncertain. We therefore show in Fig. 1 the photon spectrum at Earth with and without cascading. The full line shows the spectrum before propagation. This example is for an E^{-2} proton spectrum with a cutoff at 10^8 GeV and a total injected power of 10^{40} erg s $^{-1}$.

Our calculation shows that if a proton beam of power $\sim 10^{40}$ erg s $^{-1}$ and spectral index $\gamma \leq 2.6$ were accelerated at SN1987A, an observable γ -ray would be produced. We shall now argue that such power is achievable in young supernova remnants for a range of pulsar parameters.

The initial energy loss rate of a pulsar through magnetic dipole radiation is

$$\dot{E} = 4 \times 10^{43} B_{12}^2 P_{ms}^{-4} \text{ erg s}^{-1} \quad (1)$$

where P_{ms} is the pulsar period in ms and B_{12} is the neutron star surface field in units of 10^{12} G. As the dipole electromagnetic waves cannot propagate in the high density surrounding the pulsar¹⁰ the pulsar spin-down energy will most likely be carried off by a relativistic magnetohydrodynamic wind consisting mostly of electron-positron pairs. In their model for the Crab nebula, Rees and Gunn¹¹ proposed that the nebula could confine the wind, which would terminate at a standing shock at the point where the wind ram pressure balances the total magnetic field and particle pressure in the nebula: this would occur at a distance

$$r_s = v_{exp}^{3/2} t / \sqrt{3} c = 2.8 \times 10^{14} \tau_{mo} v_9^{3/2} \text{ cm} \quad (2)$$

where τ_{mo} is the age of the nebula in months and $v_9 = v_{exp}/10^9$ cm s $^{-1}$ is the outer expansion velocity of the nebula. Both electrons and protons could be accelerated by first-order Fermi acceleration at the shock, even though the wind itself may contain only electrons and positrons. For pure dipole spin-down, the period increases as

$$P_{ms}^2(t) = 2 \times 10^{-9} t B_{12}^2 + P_0^2 \quad (3)$$

where P_0 is the initial period of the pulsar. Defining a characteristic spin-down time $\tau_s = 16(P_{0,ms}/B_{12})^2$ yr, the total power in accelerated particles is $\dot{E}_p(t) = \epsilon \dot{E}_0/(1+t/\tau_s)^2$, where $\epsilon(t)$ is the efficiency of converting wind energy into accelerated particles. If the particle energy flux at SN1987A is bigger than the magnetic energy flux, as in the Crab nebula wind¹¹, we expect a significant fraction of the thermalized wind energy to appear in accelerated particles with spectral index ≥ 2 .

Assuming, as in the case of the Crab, that the nebular magnetic field emanates from the pulsar, the field at the shock can be found from the pulsar surface field, B_0 . If the field is dipolar ($\propto r^{-3}$) within the light cylinder and toroidal ($\propto r^{-1}$) in the wind, the magnetic field at r_s is

$$B_s = B_0 \left(\frac{r_0}{r_{LC}} \right)^3 \frac{r_{LC}}{r_s} = \frac{147 B_{12}}{P_{ms}^2 \tau_{mo} v_9^{3/2}} \quad (4)$$

where $r_0 = 10^6$ cm is the pulsar radius, $r_{LC} = c/\Omega = 5 \times 10^6$ cm arg P_{ms} is the light cylinder radius. The field at the wind shock for a millisecond pulsar is much higher than the field $\sim 10^{-4}$ G for the Crab nebula because the distance to the light cylinder, where the field falls off most rapidly, is only a few stellar radii.

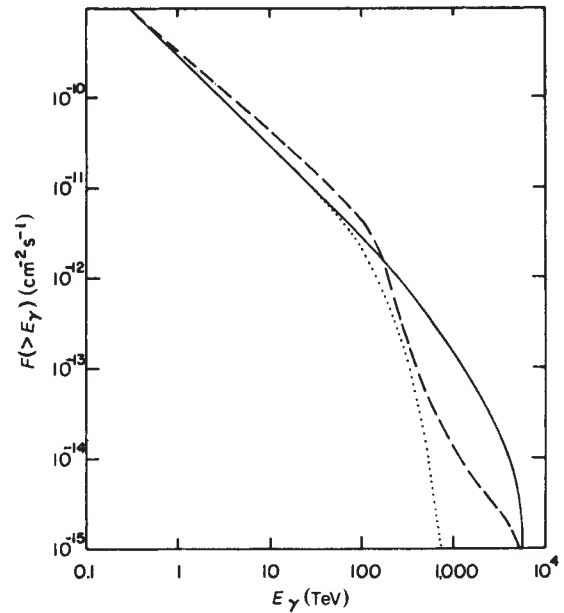


Fig. 1 Integral γ -ray fluxes at Earth from π^0 decay. The solid line shows the flux produced; dotted line excludes cascading (full absorption); dashed line includes cascading.

By equating the acceleration time¹³ and the diffusion time-scales, we obtain by equation (4) the maximum energy to which the pulsar wind shock is capable of accelerating protons:

$$E_p^{\max} = 1.9 \times 10^6 B_{12} P_{ms}^{-2} \text{ TeV} \quad (5)$$

This is an upper estimate for $E_p^{\max}$ because the minimum diffusion coefficient has been used to calculate the acceleration time. The acceleration time is inversely proportional to magnetic field at the shock, which is large see equation (4). This is what allows such large values of $E_p^{\max}$.

We expect accelerated protons (or nuclei) to be contained within the expanding shell of radius R_N for a time $\tau_e > 3R_N/c = 0.1 v_9 \tau_{mo}$, where a diffusion velocity $c/3$ corresponds to minimum diffusion coefficient. Thus all accelerated particles will be completely contained in the expanding shell if diffusion is at least ten times the minimum, which we assume to be the case. Then, until the time at which the interaction rate, $c\sigma M/(M_H^4 \pi v^3 \tau^3)$, falls below the expansion rate we can assume that all accelerated protons interact at least once. (Here, M and M_H are, respectively, the mass of the envelope and the proton mass.) After this time, only a fraction of the accelerated protons interact to produce photons and neutrinos. Thus

$$f = \min \left[40 \frac{M}{M_\odot} v_9^{-3} \tau_{mo}^{-2}, 1 \right] \quad (6)$$

is the fraction of wind power in equation (1) that emerges in photons and neutrinos. For $t > 6 v_9^{-3/2} \sqrt{M/M_\odot}$ months. Equation (6) implies that the power in proton-induced secondary particles will begin to decrease quadratically with time. The suppression factor in equation (6) must then be combined with the decrease in power due to pulsar spin-down. For t such that $f=1$, the full power of the wind could go into photons (and neutrinos) through accelerated particles. Equation (1) therefore sets the scale for estimating expected signals.

Table 1 Photon fluxes at Earth (cm $^{-2}$ s $^{-1}$)

Flux above	Spectral index, γ					
	2.0	2.2	2.4	2.6	2.8	3.0
100 MeV	2.1×10^{-6}	5.0×10^{-6}	6.7×10^{-6}	7.3×10^{-6}	7.4×10^{-6}	7.3×10^{-6}
1 GeV	3.8×10^{-7}	7.0×10^{-7}	6.3×10^{-7}	5.0×10^{-7}	3.7×10^{-7}	2.7×10^{-7}
1 TeV	3.5×10^{-10}	1.3×10^{-10}	3.0×10^{-11}	5.9×10^{-12}	1.1×10^{-12}	2.1×10^{-13}

Rees¹⁴ has recently suggested that the observed 'companion' of SN1987A indicates a super-relativistic pulsar wind escaping through a hole in the shell. Its estimated power is $10^{41} \text{ erg s}^{-1}$. If only a tenth of that power is trapped inside the nebula, it would still be sufficient to produce observable high-energy γ -ray signals.

Eichler and Letaw¹⁵ have derived limits on the power of proton acceleration in young remnants from the production of secondary nuclei in the shell. The limit depends on the effective proton energy. For $E_p^{\text{eff}} = 300 \text{ GeV}$ it is $0.03 E_{50}$, and for 30 TeV , $1.7 E_{50}$, where E_{50} is the total energy in accelerated protons in units of 10^{50} erg . For flat proton spectra this allows powers up to $10^{41} \text{ erg s}^{-1}$ for several years.

The observed signal depends not only on the rate of production of photons but also on how much they are attenuated on the way out of the production region. For a shell of mass M and uniform density the total thickness of matter in the shell becomes less than one radiation length¹ at time

$$t_\gamma = \left(\frac{M}{M_\odot} \right)^{1/2} v_9^{-1} \text{ months} \quad (7)$$

Protheroe¹⁶ has calculated the other major source of absorption-photon interactions on the thermal radiation of the nebula. For an effective shell temperature of $5,000 \text{ K}$ half the shell will become transparent to TeV photons 100 days after the explosion. In a more realistic shell model¹⁷ the density is larger in the inner, slower region than in the more rapidly expanding outer regions. This increases the optical thickness of the shell compared to one of uniform density. For example, for the model B15 of ref. 17 ($M = 15 M_\odot$) the thickness is 2 radiation lengths after 36 weeks and 1 radiation length after one year. On the other hand, the time at which adiabatic losses begin to dominate over collision losses will also be delayed, so the net effect is to delay the era of prolific secondary particle signals somewhat. In the model of ref. 17 the shell temperature is low enough that by the time the shell becomes optically thin, TeV γ -rays will not be significantly absorbed by thermal photons.

During the earlier period the density in the inner region of the shell will probably prevent the formation of the termination shock because the wind will dissipate its energy in radiation by electromagnetic cascading rather than by collisionless interaction with the nebular magnetic fields. If we define the critical time to be when the thickness of matter between the pulsar and the nominal shock position (equation (2)) is more than 1 radiation length, then the shock is established after two months in the uniform density case and after a year in the model of ref. 17. Even before that time, however, one could have some signal from the electromagnetic cascade produced by the wind particles. These questions require further investigation.

Finally we consider the contribution of electrons accelerated at the termination shock to the TeV photon flux after the collisionless shock is established. The maximum energy, E_e^{max} , to which electrons can be accelerated can be estimated by equating the energy loss and acceleration timescales. For shell temperatures $\leq 10^4 \text{ K}$ synchrotron losses dominate and we obtain

$$E_e^{\text{max}} = 17 \text{ TeV } B^{-1/2} = 1.4 \text{ TeV } P_{\text{ms}} B_{12}^{-1/2} \tau_{\text{mo}}^{1/2} v_9^{3/4} \quad (8)$$

The electron spectrum will be modified by the synchrotron losses and, for escape times much longer than the acceleration time, will exhibit a pile-up peak¹⁸ just below the electron cut-off energy E_e^{max} . We estimate the flux of synchrotron photons from the pile-up, assuming that all energy loss comes in photons of the synchrotron peak frequency of 6.4 MeV ($= \frac{1}{3} \nu_c$), which in this model is independent of all parameters and constant in time. The flux of such photons at Earth is proportional to the total dipole energy in accelerated electrons. It is

$$F(6.4 \text{ MeV}) = 12 B_{12}^2 P_{\text{ms}}^{-4} \epsilon_e \text{ photons cm}^{-2} \text{ s}^{-1} \quad (9)$$

where ϵ_e is the fraction of the dipole energy that goes into

pile-up electrons. The photon production spectrum is the single particle emissivity, which is proportional to $\nu^{1/3}$ below ν_c . Such fluxes will be easily detectable with rocket experiments in the Southern Hemisphere when the optical depth of the shell becomes smaller than unity, and may even exceed the expected line fluxes from radioactive decays¹⁷.

The synchrotron-Compton scattering model¹⁹, in which electrons boost their own synchrotron radiation, may only become applicable to SN1987A at a much later stage. Thus the $\sim \text{TeV}$ electrons of equation (9) will not make a significant contribution to the TeV γ -ray fluxes during the first few years. The observation of the energy spectrum of very high energy ($> 1 \text{ TeV}$) γ -rays would therefore be evidence that protons were being accelerated. A measurement of the spectrum of such photons would allow an estimate of the spectral index of the accelerated protons, which would reflect details of the acceleration process.

Particle acceleration in the supernova blast wave shock is inefficient at early times because a significant fraction of the kinetic energy of the expanding shell is not converted into relativistic particles until a few thousand years after the initial explosion, when the shell begins to slow down as a result of swept up interstellar gas¹⁰. Consequently, the detection of continuum TeV or PeV γ -rays from SN1987A in the first few years would be strong evidence for particle acceleration powered by a young pulsar.

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Importance of stress in bubble ordering in the helium gas-bubble superlattice in copper

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Microstructural changes induced in metals by ion bombardment have important implications for technology (see, for example, refs 1-3). Helium irradiation can result in the formation of small ($\sim 2 \text{ nm}$ diameter) helium bubbles in high concentration ($\sim 10^{25} \text{ m}^{-3}$), ordered on a superlattice. Current theories are all

directed towards explaining the formation of a superlattice having the same alignment as the crystal lattice of the metal—the matrix or m orientation. In the face-centred-cubic metal copper, although there was evidence in previous work that some domains⁴ in the bubble array were in orientations other than m (ref. 5), it may have been assumed that the proportion of such domains was small. Here we report new results that show that a high proportion of the ordered bubble array is in domains that have orientations different from m . We propose that a new mechanism, based on the spatial characteristics of the stress field around an overpressurized bubble, must play a dominant role in the later stages of bubble ordering.

It has become widely accepted that an important characteristic of cavity lattices is that the superlattice forms in the m orientation. For example, in the most recent paper on the theory of bubble ordering⁶ it is asserted that the most striking feature of the bubble lattice is that it “always has the same symmetry and alignment as the host matrix”.

The transmission electron micrograph in Fig. 1 shows bubbles typical of those found in (011)_{Cu} grains in copper following helium implantation at 300 K. Diffraction from the ordered bubble array causes the satellite spots in the electron diffraction pattern (Fig. 1, top). In previous work⁵, to explain the diffraction observed with the incident electron beam direction at $B = [011]_{\text{Cu}}$ in an (011)_{Cu} grain, it was proposed that within any given domain the bubble array should be modelled as a fully ordered face-centred-cubic superlattice, termed a structural variant, in one of three possible orientations: Δ , Ω and m (see Fig. 2). The diffraction pattern was regarded as a superposition of the patterns from the two variant structures Δ and Ω on the pattern from the variant m .

In the present work we have determined both the spatial distribution and frequency of occurrence of these variants by comparing the projected bubble images in Fig. 1 with theoretical patterns for each variant derived as in Fig. 2. These patterns were then combined to preserve bubble rows (an obvious feature of Fig. 1), as shown in Fig. 2, to find the patterns for various combinations. Analysis of a region containing 800 bubble images yields the following percentage areal coverages for the different variant structures: $m \approx 5$, $\Delta \approx 0$, $\Omega \approx 0$, $\Delta + m \approx 25$, $\Omega + m \approx 25$, $\Delta + \Omega \approx 9$, $\Delta + \Omega + m \approx 9$ and random ~ 27 . Based on projected areas, over half the ordered bubble array consists of the variants Δ or Ω , bubble lattices having orientations that differ from the m orientation assumed in all current theories of bubble ordering⁶⁻⁹. Another important finding is that the Δ or Ω structures frequently lie immediately above or below m with respect to the specimen surface, with the two domains having a common set of dense-packed $\{111\}$ bubble planes orthogonal to the grain surface.

Although the presence of the Δ and Ω orientations is not explained by existing theories, we have identified a new mechanism which, in addition to the mechanisms included in existing theories, can explain the evolution of the entire ordered bubble array including the variants Δ and Ω . Specifically, we propose that the bubbles order first into the m orientation, but that at a later stage the bubbles in some domains move to take up either the Δ or Ω orientation. For Δ and m the relative orientations of bubbles in the common $\{111\}$ bubble plane found by stereographic projection are shown in Fig. 3. For m the nearest neighbour bubbles are along $\langle 110 \rangle_{\text{Cu}}$. Note that in changing from m to Δ the separation of neighbouring bubbles increases along the three $\langle 110 \rangle_{\text{Cu}}$ directions lying in the common $\{111\}$ bubble plane. In fact it can be shown by stereographic projection that in changing from m to Δ (or Ω) the separation of neighbouring bubbles along all six $\langle 110 \rangle_{\text{Cu}}$ directions is increased (work in preparation). Why should bubbles move in this way?

The bubbles are overpressurized^{1,10}, and grow by an athermal process such as dislocation punching¹¹. An isolated bubble growing in a stress-free matrix is expected to punch out prismatic loops which move along glide cylinders in $\langle 110 \rangle_{\text{Cu}}$ directions^{12,13},

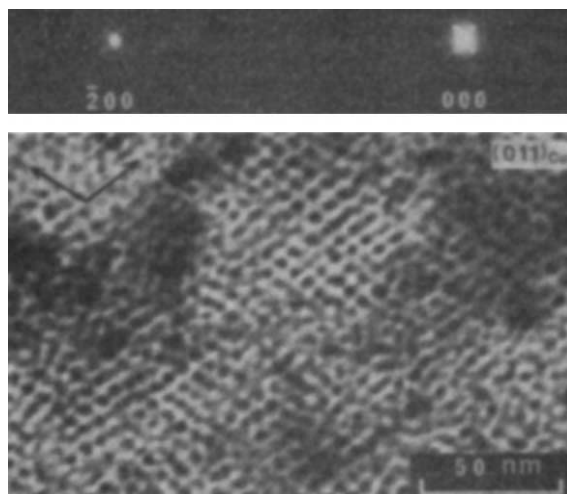


Fig. 1 A direct transmission electron micrograph (over-focus image) of helium gas bubbles in an (011)_{Cu} grain following helium-ion irradiation (4×10^{21} 30-keV He ions per m²) in the face-centred-cubic (f.c.c.) metal copper at 300 K. The electron beam is along [011] in the Cu matrix and is normal to the plane of the bubble layer. The directions of the traces (Tr) of $\{111\}$ Cu planes in the (011) plane are indicated by the arrows: Tr(111) to the left and Tr(111) to the right. Considerable bubble alignment is seen, with dense-packed $\{111\}$ bubble planes appearing edge-on as bubble rows along Tr $\{111\}$ matrix directions. A section of the selected-area diffraction pattern from the specimen is shown at the top of the figure in the correct orientation relative to the bubble array. Diffraction from the ordered bubble array causes the satellite spots evident around the (000) transmitted beam.

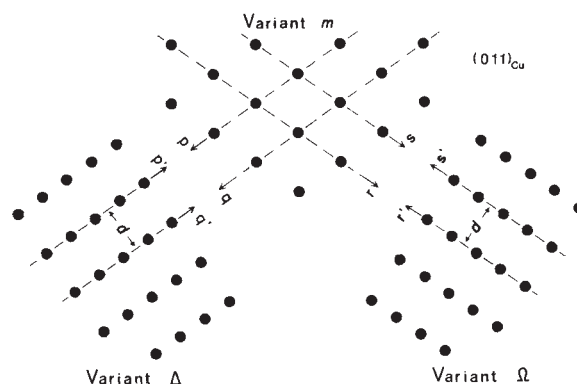


Fig. 2 Real-space theoretical bubble projections for the three variant structures Δ , m and Ω , with $B = [011]_{\text{Cu}}$. Δ , m and Ω are identical f.c.c. lattices of the same bubble-lattice parameter, $a_1 = 7.5$ nm, which differ only in orientation. The spacing between dense-packed $\{111\}$ bubble planes is denoted by d . The pattern for each variant alone was determined as the projection on (011)_{Cu} of an ideal f.c.c. bubble lattice in the appropriate orientation (as specified in ref. 5). $[112]_{\Delta}$ and $[\bar{1}12]_{\Omega}$ both coincide with $[011]_{\text{Cu}}$, which is directed out of the page. Tr(111) _{Δ} coincides with Tr(111)_{Cu} and Tr(111) _{Ω} coincides with Tr(111)_{Cu}. In forming combinations of these patterns (not shown), bubble rows have been preserved; for example, in overlaying Δ onto m to form the combined pattern $\Delta + m$, the rows p' and q' in Δ were aligned with the corresponding rows, p and q , in m to produce bubble rows in the same direction in the composite pattern.

as shown schematically in Fig. 4. Any impediment to the glide of these dislocation loops, such as the presence of another bubble on the same glide cylinder, could be expected to result in a back-stress on the bubble which will tend to inhibit bubble growth¹⁴.

Consider the interaction between two neighbouring bubbles in the particular case where the line joining their centres lies

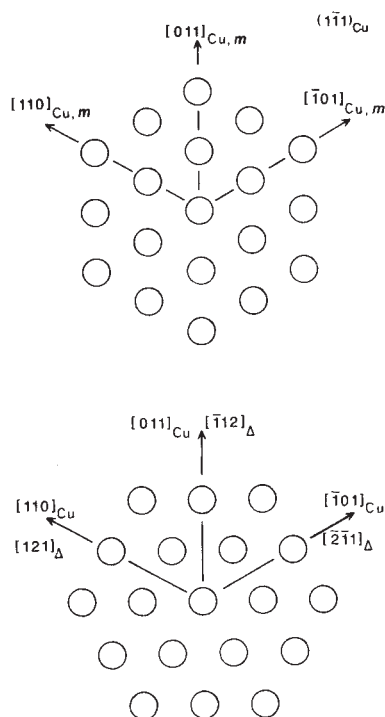


Fig. 3 Relative orientations of bubbles, for variants m and Δ , in the common bubble plane $(1\bar{1}1)_{\Delta,m}$, which is orthogonal to the grain surface and corresponds to $(1\bar{1}1)_{Cu}$. (The normal to this plane, $[1\bar{1}1]_{\Delta,m}$, is directed into the page.) The bubble density on this plane is unaltered by the change from m to Δ . (No assumption is made about the path taken by individual bubbles during the change.) In an f.c.c. lattice neighbouring bubbles are closest along $\langle 110 \rangle$. For m , the $\langle 110 \rangle_m$ are along $\langle 110 \rangle_{Cu}$, so all the $\langle 110 \rangle_{Cu}$ directions radiating out from any given bubble are blocked by nearest-neighbour bubbles. In contrast, in Δ (or Ω), the neighbouring bubbles along $\langle 110 \rangle_{Cu}$ are widely spaced. A result of the change from the m to the Δ lattice is that the spacings of neighbouring bubbles along the six $\langle 110 \rangle_{Cu}$ directions (three in the $(1\bar{1}1)_{Cu}$ plane and three directed out of this plane) are increased; there are corresponding decreases in the spacings in other directions. The same is true for the change from m to Ω . It is proposed that these increased separations result from bubble movements driven by strong bubble-bubble repulsions that develop along $\langle 110 \rangle_{Cu}$ directions at a late stage in the implantation.

along $\langle 110 \rangle_{Cu}$. The two bubbles are then linked by a common glide cylinder. Because there is a strong tendency for each bubble to punch out dislocations on this cylinder in the direction of the other, the copper matrix between the bubbles will be highly compressed. The resulting back-stresses could be expected to give rise to a repulsion between the bubbles along the common $\langle 110 \rangle_{Cu}$ direction¹⁴ (see also ref. 15). This repulsion is in addition to the attractions and the repulsions proposed in the earlier theories^{7,8}; in a recent theory⁶ it has been included as the mechanism that stabilizes a lattice in the m orientation against the coalescence of nearest-neighbour bubbles. It seems reasonable to model this repulsion as an interaction between a dislocation at the surface of one bubble and a dislocation on the same glide cylinder at the surface of the neighbouring bubble. On the basis of dislocation-dislocation interactions¹⁶ we suggest that this repulsion increases rapidly with increasing bubble size and with decrease in the spacing between bubbles (work in progress) and that it can eventually lead to the lattice becoming unstable against bubble movement.

The spacing of neighbouring bubbles along $\langle 110 \rangle_{Cu}$ is least for the variant m (Fig. 3), so particularly strong bubble-bubble

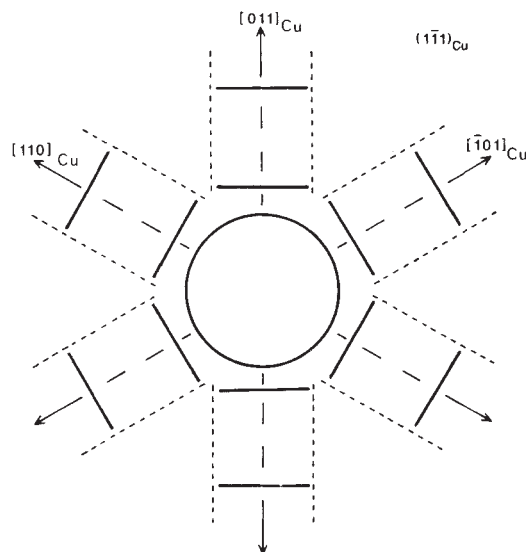


Fig. 4 An overpressurized bubble growing by dislocation punching is expected to punch out prismatic loops which lie in either $\{111\}_{Cu}$ or $\{110\}_{Cu}$ planes and move along glide cylinders in $\langle 110 \rangle_{Cu}$ directions. The glide cylinders with $\langle 110 \rangle_{Cu}$ axes lying in the $(1\bar{1}1)_{Cu}$ plane are shown schematically in the figure, with prismatic loops parallel to $\{110\}_{Cu}$ planes seen edge-on. The interaction between two bubbles lying on the same glide cylinder is expected to include a mutual repulsion which increases rapidly with increasing bubble size and with decrease in the spacing between bubbles. The spacing of neighbouring bubbles along $\langle 110 \rangle_{Cu}$ is least for the variant m , so particularly strong bubble-bubble repulsions along $\langle 110 \rangle_{Cu}$ are expected for this variant. It is proposed that these repulsions cause the bubbles in some domains to move from the m orientation to take up either the Δ or Ω configuration.

repulsions are expected in this case. We propose that in some domains these repulsions become dominant, causing the bubbles to be driven out of the m orientation and into either the Δ or Ω configuration (in which the spacings of neighbouring bubbles along $\langle 110 \rangle_{Cu}$ are greater by at least a factor of $\sqrt{3}$). The deposition of gas and damage during implantation results in the development of a biaxial compression—the lateral stress—in planes parallel with the specimen surface¹⁷. We argue (manuscript in preparation) that in the presence of a strong lateral stress the energy is minimized when all three orientations m , Δ and Ω are represented in the bubble array. We suggest that it is for this reason that the bubble lattice in some domains does not reorient but remains in the m orientation. We conclude that there is strong evidence that stress-stress interactions play an important part in the ordering of overpressurized bubbles.

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Evidence for the climatic role of marine biogenic sulphur

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Oceanic dimethylsulphide (DMS) emissions and atmospheric aerosol particle populations (condensation nuclei, CN), resolved by latitude and season, appear to be directly correlated, in that CN, as measured with a condensation nucleus counter, are high (or low) in regions where DMS fluxes and incident solar radiation are high (or low). Although it has been previously hypothesized that CN are produced from DMS^{1,2}, we report the first attempt to correlate DMS flux and CN. As the population of cloud condensation nuclei (CCN) in marine air is a subset of the CN population^{3,4}, and CCN in turn control the albedo of marine clouds^{5,6}, DMS could be involved in climate control through a cloud albedo feedback mechanism.

The transfer of solar radiative energy to the Earth drives both atmospheric and oceanic circulation. Any process that alters this radiative transfer directly affects the climate of the planet. The surface of the unfrozen oceans, covering 70% of the planet, is relatively dark and has the potential to absorb over 90% of the incident solar energy. The presence of clouds over the oceans decreases the amount of solar radiation reaching the sea surface; thus maritime clouds, through their albedo, directly influence the radiative climate of the Earth. A knowledge of the processes determining the optical properties of clouds over the oceans is critical for refining our understanding of climate and climate change.

For a given liquid water content, the optical properties of clouds are largely determined by the number of particles within the cloud⁵. Remote marine aerosol particles consist primarily of sulphates and sea-salt^{7,8}. It is generally assumed that the sub-micrometre fraction of these particles is formed by gas-to-particle conversion, and that the primary source of this sulphate is the oxidation of reduced sulphur gases emitted from the oceans^{1,2,9}. The importance of these sub-micrometre sulphate aerosol particles to the backscatter of solar radiation, as well as their role as CCN influencing the albedo of marine clouds, has led to the theory that the flux of reduced sulphur from the oceans to the atmosphere may control the climate of the Earth^{6,10}. Recent spatial and temporal estimates of the flux of biogenic sulphur from the ocean to the atmosphere^{11,12} allow an important aspect of this hypothesis to be observationally confirmed, namely the relationship of DMS flux to aerosol particle population and solar irradiance.

Marine sub-micrometre aerosol particles are composed mainly of sulphuric acid, which is only partly neutralized by ammonia^{9,13}. There are no data on the number population of non-sea-salt sulphate particles *per se*, but the total CN population over the open ocean is usually between 100 and 400 cm⁻³ and is thought to be mostly sulphate². The mass concentration of these particles appears to increase over the biologically productive regions of the ocean¹⁴ and the number population is highest during the summer months². This seasonal trend is opposite to that of the sea-salt particles, which have typical number populations at cloud height of <1.0 cm⁻³ (refs 15, 16). The origin of the sub-micrometre aerosol sulphate particles is thought to be the oxidation of reduced sulphur gases emitted from the oceans^{1,2}. DMS is the most abundant reduced sulphur compound in open-ocean surface waters^{11,17} and is the only

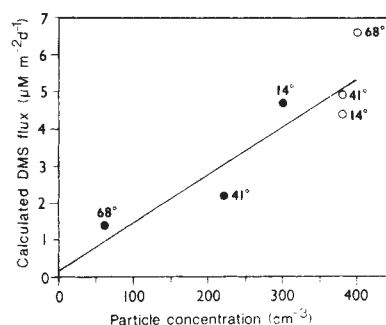


Fig. 1 Plot of calculated DMS flux¹¹ in $\mu\text{M m}^{-2} \text{d}^{-1}$ against particle (CN) concentration² in cm^{-3} . The six points represent two seasons (summer, open circles; winter, filled circles) and three latitudes (14°, 41° and 68–69°, corresponding to DMS tropical, temperate and subpolar regions). The equation of this line is $\text{flux} = 0.013 (\text{particle concentration}) + 0.22$, with a correlation coefficient, r , of 0.90. The particle data are from the Southern Hemisphere while the DMS data are from the Northern Hemisphere.

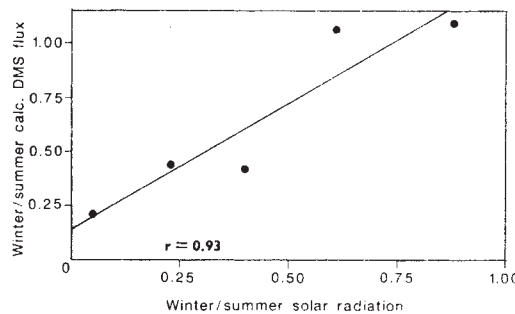


Fig. 2 Plot of the ratio of winter to summer calculated DMS fluxes in each latitudinal region (0–5°, 5–20°, 20–35°, 35–50° and 50–65° N; ref. 11) against the ratio of winter (December) to summer (June) direct solar radiation reaching the Earth. The equation of this line is $\text{flux ratio} = 1.16 (\text{radiation ratio}) + 0.16$, with a correlation coefficient, r , of 0.93. A similar relation was obtained for particle concentration and solar radiation².

significant source of reduced sulphur to the marine atmosphere¹⁸. DMS is produced by phytoplankton in the ocean's photic zone. The measured mean concentration of DMS in the atmosphere is quite low (3–12 nM m⁻³, ref. 19) resulting in a net flux of DMS from the ocean to the atmosphere. The magnitude of this flux varies with both latitude and season, and for the non-coastal ocean ranges from 1.0 to 6.0 $\mu\text{M m}^{-2} \text{d}^{-1}$ (ref. 11). The concentrations of the atmospheric oxidation products of DMS, methane sulphonic acid (MSA) and sulphate, have been shown to vary seasonally^{20–22}, although the data are conflicting.

Non-nucleated sub-micrometre sulphate aerosols contribute directly to the backscatter of solar radiation and hence act to cool the surface of the Earth¹⁰. The net cooling effect at present, though, is smaller than the heating caused by the greenhouse effect of carbon dioxide²³. Sub-micrometre sulphate particles, however, also serve as cloud condensation nuclei (CCN)²⁴ and in this capacity have a much greater effect on planetary albedo than the direct radiative effect of non-nucleated aerosols⁶. It is commonly believed that the concentration of CN is much greater than the concentration of CCN²⁵, but in the remote marine troposphere, where the CN population is small (100–300 cm⁻³), the CN and CCN populations at 0.8% supersaturation converge^{3,4, 26}. The number of sub-micrometre sulphate particles acting as CCN, therefore, is limited by the total particle population, and a change in CN population should have a direct effect on the optical properties of marine clouds. An increase in the population of CCN in marine stratus and altostratus clouds of

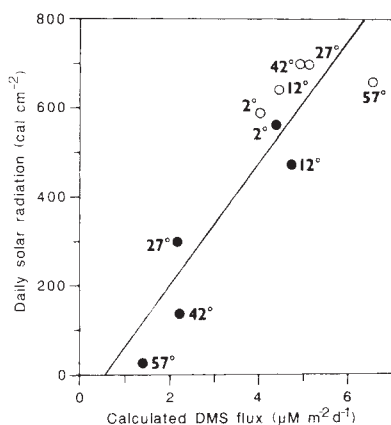


Fig. 3 Plot of calculated DMS flux¹¹ in $\mu\text{M m}^{-2} \text{d}^{-1}$ against daily direct solar radiation reaching the surface of the Earth in cal cm^{-2} . The ten points represent five latitudinal regions in two seasons (summer, open circles; winter, filled circles). The equation of this line is $\text{radiation} = 137 (\text{flux}) - 68$ with a correlation coefficient, r , of 0.90.

only 30% is calculated to increase cloud albedo by 0.02 and decrease the Earth's surface temperature by 1.3°C (ref. 6). Variations in the optical properties of maritime clouds could therefore dramatically alter the amount of heat absorbed by the ocean, and hence could have an important function in regulating the climate of the Earth.

The hypothesis, therefore, is that biogenic DMS is emitted from the ocean and transformed in the atmosphere by gas-to-particle conversion to sub-micrometre aerosol particles. These particles act as CCN and subsequently control the albedo of clouds. This hypothesis implies a direct relation between DMS flux, CN population and CCN population. Elliott & Egami³ and Hoppel⁴ have demonstrated the high correlation between CN and CCN over the remote ocean.

There have been as yet no simultaneous observations of DMS concentrations and particle number concentrations, but DMS fluxes calculated from seawater DMS measurements in the North Pacific Ocean¹¹ can be related by latitude and season to total particle concentrations at four remote Southern Hemisphere sites². Although the DMS data are from individual cruises throughout the Pacific Ocean, as opposed to year-long time-series at any one location, both the DMS flux and the particle number concentration vary seasonally and are highest in the summer. The amplitude of this seasonal variation increases with increasing latitude. A plot of DMS flux against particle number concentration for three latitudes (14° , 41° and $68\text{--}69^\circ$ corresponding to the tropical, temperate and subpolar region DMS data) and two seasons (winter and summer) shows a close linear relationship ($r=0.90$, Fig. 1). Despite a paucity of points, this correlation is surprising, considering that the comparison is based on regional average DMS data in the Northern Hemisphere compared with continuous particle data from the Southern Hemisphere. There is another way to regress these data because the relative changes in particle concentration correlate well with the relative changes in net solar radiation at each station². Regressing the ratio of winter to summer DMS fluxes to the ratio of winter to summer direct solar radiation reaching the surface of the Earth (Smithsonian Meteorological Tables) yields $r=0.93$ (Fig. 2). This adds support to the linear relation of DMS flux to particle number concentration. Bigg *et al.*² attribute the seasonal cycle of particle number concentration to the amount of radiation available for photochemical gas-to-particle transformations. The DMS flux/particle number relation shown in Fig. 1 suggests that the seasonality of particle number concentration could be attributable both to the seasonality of the marine sulphur source and to the amount of solar

radiation available for photochemical gas-to-particle conversion.

To extend this apparent DMS flux/particle-number correlation to a convincing argument for a bio-controlled thermostat of the planet requires the identification of a flux/climate relation or feedback. One obvious choice would be a temperature/flux correlation. The flux of reduced sulphur compounds from soils and plants to the continental atmosphere appears to vary directly with temperature²⁷. The average seasonal variation of temperature of the near-surface ocean is, however, much less than that of the continental surface¹¹. The correlation coefficient for this regression is therefore low ($r=0.44$), indicating that variations in ocean surface temperature have little effect on the observed seasonal flux of sulphur from the ocean to the atmosphere.

Another climatic variable that could affect the flux of DMS is incident solar radiation. The relative seasonal change in solar radiation correlates well with the relative seasonal change in both DMS flux (Fig. 2) and particle number². A plot of DMS flux in the North Pacific Ocean against daily direct solar radiation reaching the sea surface (Smithsonian Meteorological Tables, with a transmission coefficient of 0.8) for five latitudinal regions ($0\text{--}5^\circ$, $5\text{--}20^\circ$, $20\text{--}35^\circ$, $35\text{--}50^\circ$ and $50\text{--}65^\circ\text{N}$; ref. 11) and two seasons (winter DMS with December radiation, and summer DMS with June radiation) shows a linear relation with $r=0.90$ (Fig. 3). This suggests that the flux of sulphur from the ocean to the atmosphere is linearly dependent upon the amount of solar radiation reaching the sea surface.

Is it possible that marine plankton regulate the production of DMS as part of a global biological system to control the amount of sunlight reaching the Earth? During the winter at high latitudes, when the incident solar radiation is low, very little DMS is produced. The lower concentrations of sulphur in the atmosphere should result in low concentrations of sub-aerosol particles. As there are fewer particles to act as CCN and increase the albedo of clouds, the oceans should absorb more solar energy. During the summer months, when the daily solar radiation is great, plankton produce more DMS, which should subsequently produce more aerosol particles, which in turn, through CCN, should increase the albedo of the Earth. In low latitudes, where the incident radiation remains fairly constant throughout the year, the flux of DMS also remains rather constant. Even during the 1983 El Niño/southern oscillation when marine productivity along the Equator decreased by a factor of 5–10 (ref. 28), the measured concentration of DMS¹¹ remained surprisingly constant. The co-varying spatial and temporal variations in DMS flux, sub-micrometre aerosol particle concentration and solar radiation are necessary but not sufficient conditions to prove the theory of climate regulation by biospheric trace gases and cloud-albedo feedback⁶. To assess this intriguing hypothesis fully, simultaneous DMS, CN, CCN and radiation measurements in different seasons and latitudes are needed, as well as a complete understanding of the microbiological and cloud microphysical processes involved.

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Boreal forests and atmosphere-biosphere exchange of carbon dioxide

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The response of vegetation growth to fluctuations in climate or anthropogenic influences is an important consideration in the evaluation of the contribution of land biota to atmospheric CO₂ variations. Here we present two approaches to investigate the role of boreal forests in the global carbon cycle. First, a tracer transport model which incorporates the normalized-difference vegetation index (NDVI) obtained from advanced very high resolution radiometer (AVHRR) radiances was used to simulate the annual cycle of CO₂ in the atmosphere. Results indicate that the seasonal growth of the combined boreal forests of North America and Eurasia accounts for about 50% of the mean seasonal CO₂ amplitude recorded at Pt Barrow, Alaska (71° N, 157° W) and about 30% of the more globally representative CO₂ signal at Mauna Loa, Hawaii (20° N, 156° W). Second, tree-ring width data from four boreal treeline sites in northern Canada were positively correlated with Pt Barrow CO₂ drawdown (that is, maximum-minimum CO₂ concentration) for the period 1971-1982. These results suggest that large-scale changes in the growth of boreal forests may be contributing to the observed increasing trend in CO₂ amplitude. They further suggest that tree-ring data may be applicable as indices for CO₂ uptake and remote sensing estimates of photosynthetic activity.

Seasonal oscillations of atmospheric CO₂ recorded at Northern Hemisphere stations are caused primarily by the seasonal dynamics (photosynthesis, respiration and decomposition) of the terrestrial biosphere^{1,2}. These oscillations, or amplitudes, demonstrate both interannual variations and a trend towards increasing seasonal amplitude with time³⁻⁹, and both variations may be related to fluctuations in the growth of land plants³⁻⁹. It has been suggested that anomalous seasonal amplitudes might have been caused by the adverse effects of drought on plant growth in Eurasia in 1975¹⁰ and in North America in 1980¹¹. Increased metabolic activity of land plants, resulting from climatic change and/or the direct effects of CO₂ or other nutrient fertilization, may be contributing to the positive trend in amplitude, which is most pronounced at northern latitudes (that is ~1% yr⁻¹ at Pt Barrow^{7,8}). Other possible contributing factors include changes in the seasonality and transport of fossil fuel combustion, changes in atmospheric circulation, increased ocean productivity and land-use changes related to forest regrowth^{4,7,8,12,13}.

The northern boreal forests are important to the study of CO₂-vegetation interaction due to their large biomass and net

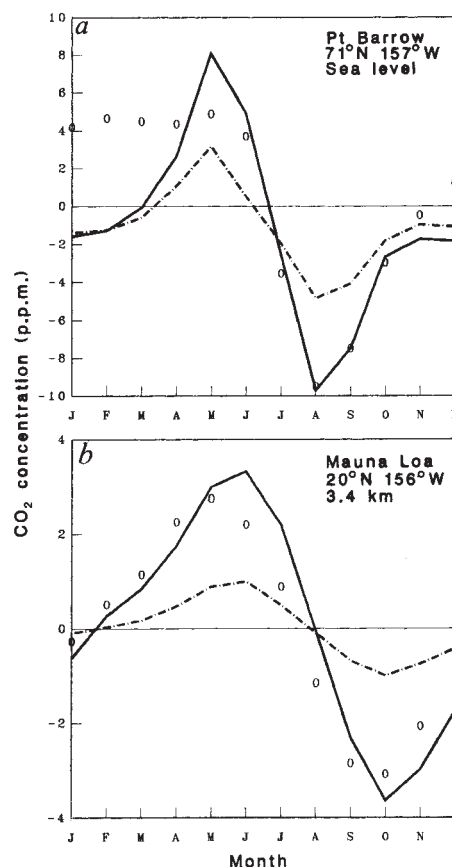


Fig. 1 Comparison of boreal (dashed line) and total (solid line) modelled annual cycles of atmospheric CO₂ at: a, Pt Barrow, Alaska, where it can be seen that the boreal portion is about 50% of the total amplitude; and b, Mauna Loa, Hawaii, where the boreal portion is about 30% of the total amplitude. Circles are observed values.

primary productivity. Unlike tropical forests, the seasons of net photosynthesis (net CO₂ uptake) and net respiration (net CO₂ release) are out of phase at high northern latitudes, and this asynchrony is reflected in the oscillations of atmospheric CO₂. The northern forests (latitude band 50°-70° N) also dominate the seasonal CO₂ drawdown for the globe^{10,14}. It therefore seems reasonable that any changes in the seasonality of CO₂ (particularly at northern latitude stations) might reflect changes in the seasonally-variable growth of the boreal forests.

Much of the uncertainty in our understanding of atmospheric CO₂ changes has resulted from a lack of appropriate indices with which to characterize the boreal forests and other components of the biosphere⁹. The recently developed NDVI, which can monitor global-scale variations in photosynthetic activity¹⁵⁻¹⁷, is a major step towards demonstrating the influence of the terrestrial biosphere on atmospheric CO₂. The inverse relationship found by Tucker *et al.*¹⁷ between this index and atmospheric CO₂ concentration during the growing season indicates that the NDVI can be used as a measure of CO₂ uptake by the biosphere. These results suggest that a long record of NDVIs may provide information about interannual and longer term changes in photosynthetic activity, and the relationship between these changes and observed variations in atmospheric CO₂ oscillations. However, as the NDVI data have only been available since 1982, other ground-based field observations must be found.

We investigated the contribution of boreal vegetation to the total seasonal CO₂ amplitudes at Pt Barrow and Mauna Loa using a 3D atmospheric tracer transport model^{13,18}. This model uses winds generated by a global general circulation model

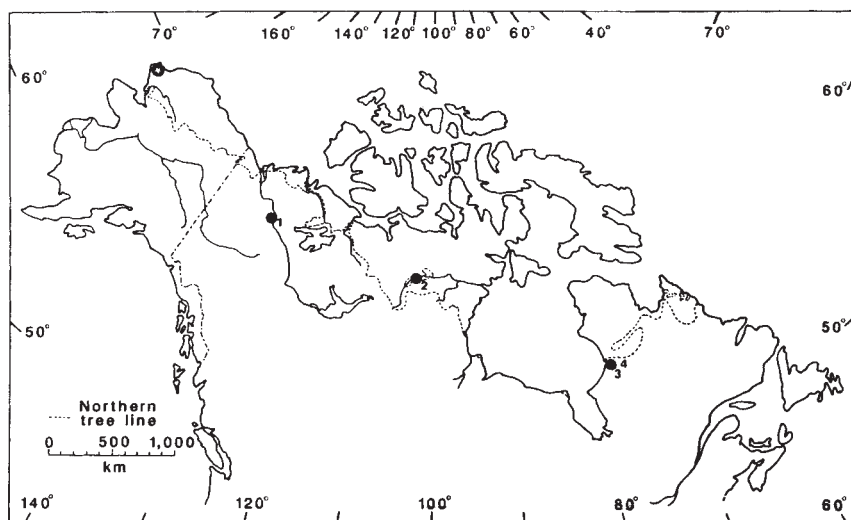


Fig. 2 Map showing North American boreal forest treeline. Dots are sites of four prewhitened white spruce (*Picea glauca*) tree-ring chronologies developed by our laboratory and used in this study: (1) Mackenzie Mt (North-west Territories); (2) Hornby Cabin (North-west Territories); (3) Cri Lake (Quebec); and (4) Cape (Quebec). Starred site is Pt Barrow, Alaska.

(GCM)¹⁹ to advect CO₂ as an inert tracer. The version of the model used for this study uses winds and subgrid-scale convective fluxes from the fine-grid (4° latitude × 5° longitude) version of the GCM. All physical processes in the fine-grid GCM are identical to those described by Hansen *et al.*¹⁹. This GCM has improved simulation of the strength and position of the Hadley circulation, synoptic-scale features as well as the higher-moment statistics of the general circulation. Atmosphere-land biosphere CO₂ exchange was modelled using monthly NDVI, soil respiration data and estimates of net annual productivity for all vegetation types. This exchange function (which ignores oceanic and fossil fuel contributions to seasonal CO₂ amplitudes) produced, in the 3D model, annual average oscillations of atmospheric CO₂ similar to those observed at stations throughout the globe¹⁸. To isolate the contribution of boreal tree growth, only the CO₂ exchange function of boreal forest vegetation was incorporated into the tracer model. The results indicate (Fig. 1a and b) that boreal vegetation growth has an important influence on seasonal CO₂ variations, particularly at high northern latitudes.

Next we sought relationships between atmospheric CO₂ variations and tree rings. Tree-ring chronologies (dimensionless indices standardized to enhance the common signal and average out noise due to endogenous factors²⁰) have been shown to be good indicators of summer temperature, but can also integrate climatic conditions throughout the year (including soil temperature and direct insolation, for example²¹⁻²³). Four pre-whitened (to remove autocorrelation effects) white spruce (*Picea glauca* (Moench) Voss) chronologies from boreal treeline sites (Fig. 2) were compared to CO₂ drawdown at Pt Barrow, Alaska. Biweekly CO₂ flask data for Pt Barrow for the 1971-1982 period were obtained from the Global Monitoring for Climatic Change (GMCC) programme of the National Oceanic and Atmospheric Administration (NOAA)⁶. These CO₂ data were detrended by removing an exponential increase due to fossil fuel input³, and the annual CO₂ drawdown (maximum-minimum) was calculated from the detrended data. Other methods of detrending (complex demodulation⁹ and bandpass filtering²⁴) produced similar interannual variations of the drawdown. Autocorrelation in the CO₂ drawdown data is nearly zero and would not affect the results presented here. Simple correlations (*r*) between these four chronologies (see Fig. 2) and Pt Barrow CO₂ drawdown are: 0.62, 0.53, 0.59 and 0.64 for Mack. Mt., Hornby, Cape and Cri Lake, respectively. All four correlations are statistically significant at the 95% level, using a one-tailed test. Figure 3 shows there is good agreement between actual CO₂ drawdown and that estimated (using multiple linear regression analysis) from the tree-ring chronologies. To substantiate further the CO₂ drawdown-tree growth link, we have calculated the prediction-*R*² (*R*_p²) statistic based on computing the prediction error sums

of squares, using the 'leave one out' method²⁵. A value of 0.64 was obtained, suggesting that the true explained variance is about 64%. Although the number of years of overlapping records presently available for analysis are limited (the calibrated Pt Barrow CO₂ record extends from 1971 to the present; most other tree-ring chronologies were sampled in the mid to late 1970s or earlier), these results are encouraging and demonstrate that the information contained in tree-ring time series may be useful as indicators of the contribution of the land biota to seasonal variations in atmospheric CO₂.

The boreal forests play a crucial part in atmosphere-biosphere exchange of CO₂. They are located in regions where the largest changes in surface air temperatures for the past century have been recorded, and where the largest greenhouse warming for doubled CO₂ concentrations has been predicted^{26,27}. The boreal forests also comprise a large proportion of the seasonally changing biomass of the terrestrial biosphere¹⁰. Tucker *et al.*¹⁷ demonstrated that the variations in NDVI from 50° to 80° N are related to CO₂ oscillations at Pt Barrow. Here we show that the rate at which CO₂ is removed from the atmosphere, as recorded at Pt Barrow, Alaska, can be positively correlated with variations in tree-ring indices from boreal sites in North America.

Based on the results of our 3D model and on the demonstrated relationship between tree-ring indices, favourable environmental conditions and CO₂ drawdown, we postulate that recent increases in CO₂ amplitudes may have been caused (at least in part) by seasonally enhanced growth of the boreal forests. Many of our tree-ring chronologies from northern Canada and Alaska demonstrate a low-frequency correspondence to the general upward trend (and occasional downward inflections) in Northern Hemisphere surface air temperatures over the past century²⁸. The direct effect of CO₂ fertilization in tree rings is still strongly debated²⁹⁻³¹. In northern ecosystems, any effect of direct CO₂ fertilization on tree growth has not yet been quantified. Although

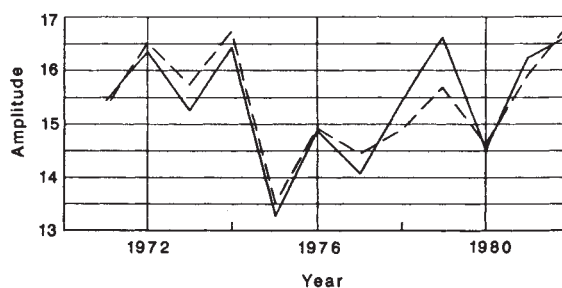


Fig. 3 Actual (solid line) and estimated (dashed line) detrended Barrow CO₂ drawdown (in p.p.m.) using four tree-ring width chronologies as predictors.

nutrient limitations may minimize such a response in these regions³², experiments have shown that a response to CO₂ fertilization can occur even in the presence of other stressfully limiting factors³³. Recent increases in anthropogenically-related nutrients³⁴ might also enhance growth in these forests.

Our results suggest a strategy for direct estimates of changes in photosynthetic activity of the boreal forests. Contemporary variations of photosynthetic activity may be provided by current remote sensing measurements, while the history of boreal forest growth may be estimated from tree-ring data. The atmospheric CO₂ record will provide correlative evidence of the estimated changes for the period overlapping the satellite and tree-ring data.

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Occurrence of a predicted earthquake on the San Andreas fault

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In May 1985 we predicted¹ that an earthquake would occur on the San Andreas fault near Stone Canyon, California within a year. The prediction was based on the observation of seismic quiescence—defined as a significant decrease in the average occurrence rate of earthquakes within the source volume of the future mainshock. A mainshock of magnitude $M_L = 4.6$ occurred on 31 May 1986, rupturing exactly the specified segment of the fault. This is the first successful prediction of an earthquake along the San Andreas fault, and the probability to have come true by chance is $<5\%$. Although the prediction addressed only a small mainshock, its success was significant because the location, size and occurrence time were correctly specified for an earthquake in a populated area. Larger earthquakes will undoubtedly be successfully predicted by the same method in the future, but the major segments of the San Andreas fault near San Francisco and Los Angeles have such a low-background seismicity rate that the method will probably not be applicable there.

The hypothesis that quiescence precedes large mainshocks²⁻⁴ is supported by 13 cases from areas other than the San Andreas fault, in which decreases in precursory rate have been measured quantitatively. The mainshock magnitudes range from 6.3 to 8.0, with shorter durations of quiescence for the smaller shocks (2-3 yr) and longer durations (4-5 yr) for the larger ones. The decrease of seismicity rate ranges from 45 to 90% (ref. 5). For the San Andreas fault system we now have four examples of precursory quiescence for events of magnitude 4.6-6.1.

Very few attempts to predict earthquakes were made by western scientists during the past decade. Using the quiescence

hypothesis, Ohtake *et al.*³ predicted the location and magnitude of the 1978 ($M_S = 7.4$) Oaxaca, Mexico, earthquake, and Kisslinger *et al.*⁶ attempted to predict the 1986 ($M_S = 7.5$) Andreanof Islands earthquake. Based on regular recurrence times, moderate mainshocks have been predicted to occur at Parkfield, California⁷, and Kaoiki, Hawaii⁸.

For the area near Stone Canyon, we noticed in the spring of 1985 that a segment of the San Andreas fault between 36.583° and 36.657° N (polygon 386, Fig. 1) had not shown a constant seismicity rate¹, unlike most neighbouring fault segments (Fig. 2a,b). A sequence of four mainshocks with equivalent magnitude $M_L = 4.9$, which occurred on both sides of, and within volume 386, perturbed the seismicity rate in 1982. Elsewhere⁹ we show that precursory quiescence preceded these events and an increase in background rate followed them. The rate increase after these shocks is clearly visible in Fig. 2a.

In spite of these perturbations we were able to demonstrate¹ in 1985 that the rate decrease in volume 386 at the end of the then available data (Fig. 2a) was unique, and hence highly significant. Uniqueness was established in the revised but unpublished version of our prediction as follows. First the minimum magnitude of homogenous reporting¹⁰ was established by the magnitude signature technique¹¹; then the dependent earthquakes (aftershocks, foreshocks, clusters and doublets) were removed¹²; finally the seismicity pattern in the target volume was compared with that in neighbouring fault segments. A detailed comparison is necessary because artificially introduced rate changes exist in seismicity catalogues. If the target anomaly was found to extend over a large portion of the area covered by the central California seismograph network one might doubt the tectonic origin of the anomaly, however, if only a short fault segment shows a rate decrease, then it is likely that the change is due to tectonic processes. For comparison with the anomalous target volume (386) we chose the 100-km segment of the San Andreas fault south of 36.95° N, which includes the target volume. This arbitrarily chosen segment of the fault was subdivided into 39 5-km segments, such that each overlapped adjacent ones by 50%. Then we moved an 89-week window (the duration of the anomaly at the time) by one-week steps through

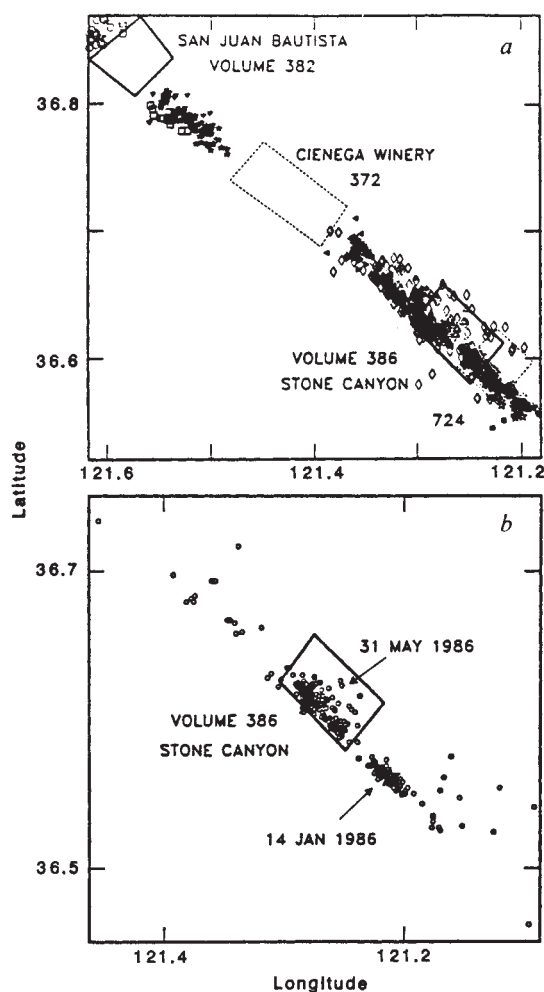


Fig. 1 *a*, Map of a section of the San Andreas fault showing aftershocks (two weeks with different symbols for each case) for mainshocks between August 1974 and January 1985. Solid polygons define crustal volumes of depth 20 km in which mainshocks were predicted. Note that absence of aftershock zones is defining a seismic gap near the Cienega Winery, however, precursory quiescence can occur on any segment of the fault, regardless of its background seismicity rate or aftershock activity. *b*, Map of the aftershock areas of the predicted Stone Canyon earthquake of 31 May ($M_L = 4.6$), and the missed mainshock of 14 January 1986 ($M_L = 4.6$). Plotted are all events which occurred within 14 days of these mainshocks. The polygon number 386 defined the location of the predicted event a year before it occurred.

the seismicity data for each of the 5-km segments, comparing each 89-week average rate with the overall average for the subsegment. For evaluating the difference in average rates we used the standard deviate z -test^{10,11}. The z -value is zero if there is no difference between the two averages; the larger the z -value, the more significant is the difference.

The histogram resulting from 7912 comparisons of the averages (Fig. 3) shows the expected peak near $z = 0$, and has a standard deviation of 0.93. Although the observed distribution of z -values failed the χ^2 test for normality at the 90% confidence level, we will assume that the z -test is a measure of the degree of difference between the two averages. In Fig. 3 the arrows point to the z -values calculated for the target period in segment 386 ($z = 3.1$ and $z = 4.6$ for $M_L \geq 1.7$ and $M_L \geq 2.0$, respectively). Both of these values are more than three standard deviations from the mean, and both of them are unsurpassed by any other rate comparison. Based on this observation of an uniquely strong

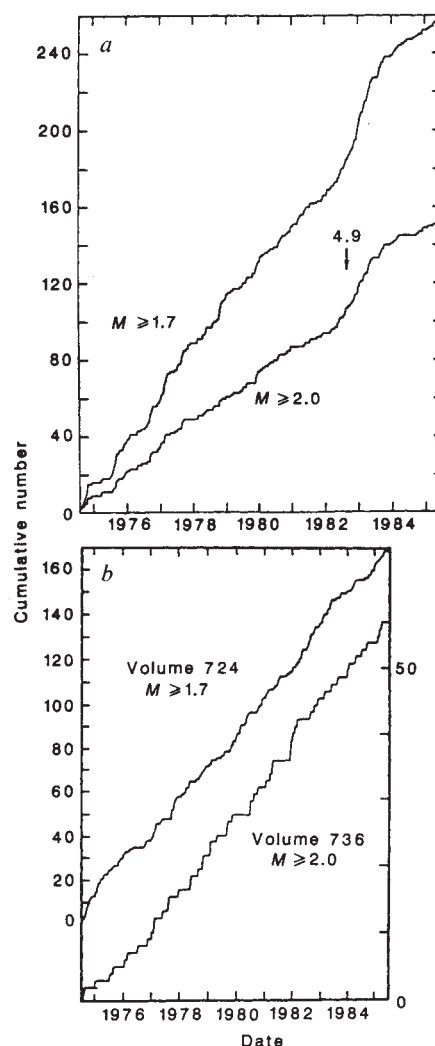


Fig. 2 *a*, Cumulative number of earthquakes as a function of time for polygon 386 defined in Fig. 1. The two curves are for events of $M_L \geq 1.7$ and $M_L \geq 2.0$. The occurrence time of the 1982 mainshocks ($M_L = 4.9$ is the equivalent magnitude of the cluster) is marked by the arrow. The slope of these curves equals the seismicity rate and is noticeably lower during the last two years of the data. A prediction of a mainshock was issued based on this data set. *b*, Cumulative number of earthquakes that occurred within two randomly chosen volumes of length 5 km located on the San Andreas fault near the target volume. In these volumes the seismicity rate is more nearly constant throughout. The location of volume 724 is given in Fig. 1*a*, 736 is located at latitude 36.38° N.

rate decrease, we proposed that this anomaly should be interpreted as an earthquake precursor. The possibility that the quiescence anomaly was artificially introduced into the earthquake catalogue was discounted because neighbouring fault segments of 5–10 km did not show it.

At the 27 July 1985 meeting of the National Earthquake Prediction Evaluation Council (NEPEC) we identified three subsegments (5–10 km long) of the fault which appeared to be quiet. We offered two interpretations for this observation: either the three quiescences could have been related to the preparation process of a single, fairly large earthquake; or each segment was to break separately by mainshocks of magnitude 4.5–5.5. Preference was given to the latter because the seismic history of this part of the San Andreas fault does not include ruptures larger than $M_L = 5.75$. After the NEPEC review we re-analysed the data, responding to several points of criticism, and we withdrew one of the predictions, because the anomaly in that

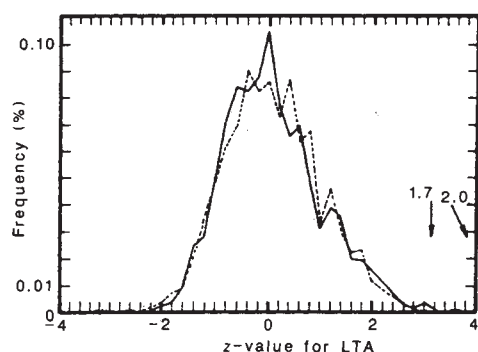


Fig. 3 Histogram for z -values resulting from 7,912 comparisons of the long-term average (LTA) rate with the rates in 89-week windows in 5-km segments of the San Andreas fault south of 36.95°N . The solid and dotted lines are for $M_L \geq 1.7$ and $M_L \geq 2.0$ respectively. Arrows point to the z -values for the target period in volume 386 which reach values of 3.1 and 4.6 ($M \geq 1.7$ and $M \geq 2.0$ respectively), when one standard deviation from the mean equals 1.

segment (dashed line in Fig. 1a) was not statistically as highly significant as the others. We confirmed that the other two anomalies (in volumes 382 and 386, Fig. 1a) were extremely unusual, and we retained the interpretation that these segments were likely to rupture within a year of May 1985.

On 14 January 1986 an earthquake of $M_L = 4.6$ occurred in Bear Valley, 5 km south of segment 386 identified as anomalous (Fig. 1b). At first it seemed that this might have been the predicted mainshock, because the initiation of rupture sometimes is located outside but near the quiet volume^{8,13}. But the aftershocks of this earthquake did not extend into the target volume, so it was not a fulfillment of the prediction. Such a case, where a mainshock occurs which was not predicted but had a magnitude similar to a predicted event, and was located within the area studied is called a 'failure to predict'.

On 31 May 1986 another mainshock of $M_L = 4.6$ occurred in the area. This time the epicenter was located within our target area, and the aftershocks extended almost exactly over the fault segment specified by us (Fig. 1b). From the extent of the aftershocks we estimate that the rupture length was only 10% shorter than predicted. An excellent agreement, considering that a factor of two would be acceptable. We regard this as a fulfillment of our prediction of that segment of the fault. No earthquake of the predicted magnitude occurred within the other area we had designated (polygon 382) during the year covered by our prediction, so this is regarded as a false alarm.

For assessing the usefulness of a predictive tool one needs to estimate the false-alarm rate as well as the failure rate. Unfortunately these are not known reliably for any earthquake precursors. Although we have searched for false alarms and failures systematically in the case of quiescence^{8,14,15} we have not enough data to establish these rates. Our preliminary estimate is that they may be ~50%. In the present case of a 100-km study segment along the San Andreas fault we have one success (the predicted 31 May 1986 mainshock), one failure (the 14 January 1986 event), and one false alarm (the prediction of a mainshock near San Juan, Fig. 1a, was not fulfilled). Quiescence as tool for earthquake prediction would clearly be useful if this observation should turn out to be representative for the false alarm and failure rate along the San Andreas fault system.

The probability that the May 1986 Stone Canyon mainshock fulfilled our prediction by chance is estimated as <5%, based on the following. We divided a 100-km study segment of the fault into 20 segments of equal length, and predicted mainshocks in two of those. Thus the probability for a mainshock to occur within the time window at random in either of the specified locations is 1/10 of the frequency of occurrence. The frequency

of earthquakes that qualify as successes is defined as the number of events during the data period studied by us (August 1974–May 1985), for which the length of the two-week aftershock area exceeds half of the length of the target segment 386, divided by the duration of the data period. With this definition we find two qualifying events in 11 years, resulting in a frequency of 2/11 per year. As our time window for the prediction was one year we find the probability of chance success to be $1/10 \times 2/11 = 2\%$. If one extends the definition of qualifying mainshocks to three additional quakes which were almost large enough to qualify, then one obtains the maximal reasonable chance probability to be 4.5%.

An even more compelling reason for believing that the seismic quiescence was a real precursor, comes from the observation of fault creep retardation in Stone Canyon¹⁶. The studied fault segment of the San Andreas is known for a seismic displacement steps of amplitude of a few millimetres, which occur fairly regularly and are called creep events. The creep rate is smallest in the north and decreases towards the south of the studied fault segment, but it remains constant over many years at six locations where it is measured¹⁶. The creep meter at Lewis Ranch, which is located at the northern limit of the target polygon 386, showed a decrease of creep rate by about 50%¹⁶ starting in 1983. This observation prompted the analysis of seismicity patterns along this fault segment, which led to our predictions¹. Other creep meters, except possibly the one near San Juan¹⁶, did not show creep retardations. Hence we propose that seismic quiescence and creep retardation are both expressions of the preparation process for mainshocks along this segment of the San Andreas fault. One may speculate that the fault gets stuck by some unknown process during the three year precursor time, and then catches up by slipping in a small mainshock.

We conclude that the fulfillment of our prediction of the 31 May Stone Canyon earthquake was most likely not a chance occurrence. Based on the observation that some moderate and large shocks have been preceded by clear seismicity quiescences we believe that the quiescence hypothesis is applicable to earthquake prediction. Considering that we proposed a correct prediction, together with an alternate scenario for the Stone Canyon earthquake, we see the need for refining the quiescence hypothesis. The precursor time versus magnitude relation especially needs to be defined better, and perhaps separately for different tectonic areas. From the amount and vigour of criticism we received during the year in which this prediction of a small earthquake was in effect we conclude that the pre-event discussion for a large shock might be so heated as to be counter-productive for earthquake hazard mitigation. But it seems worthwhile to learn how to interpret seismic quiescence before the mainshock has occurred, and to deal with earthquake predictions when probability of fulfillment is poorly known.

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Large-scale biogeographical patterns of species richness of trees

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Biologists have long recognized the striking geographical variability of species richness¹. A primary goal of contemporary ecology is to identify the factors responsible for this variability². We have examined the spatial distributions of trees in North America to determine which characteristics of the environment are most closely related to the species richness of different regions. Realized annual evapotranspiration, which is correlated with primary production and is therefore a measure of available energy, statistically explains 76% of the variation in species richness. Topography and proximity to the sea are significantly related to the residual variation, whereas seasonal climatic variability and glacial history are not. Tree richness in Great Britain and Ireland can be accurately predicted from these North American patterns. Our data are best explained by the hypothesis that contemporary available energy limits species richness^{3,4}.

Studies of several groups of animals have identified pronounced latitudinal gradients in species richness, as well as greater richness in mountainous areas, fewer species on peninsulas, and certain longitudinal trends⁵⁻⁷. Many hypotheses have been proposed to explain this spatial variability in species richness. Richness has been postulated to depend upon historical factors (such as speciation and dispersal), climate, climatic variability, topography, biotic processes (primary productivity, competition and so on), disturbance, and the richness of other groups of organisms⁵⁻⁹. Many of these factors undeniably operate on the local scale¹⁰⁻¹². The present study considered the variability of species richness among regions and sought to identify the factors most closely related to it.

We examined the distributions of North American trees using methods similar to those of Simpson⁵. The continental United States and Canada were divided into 336 quadrats, each $2\frac{1}{2}^\circ \times 2\frac{1}{2}^\circ$

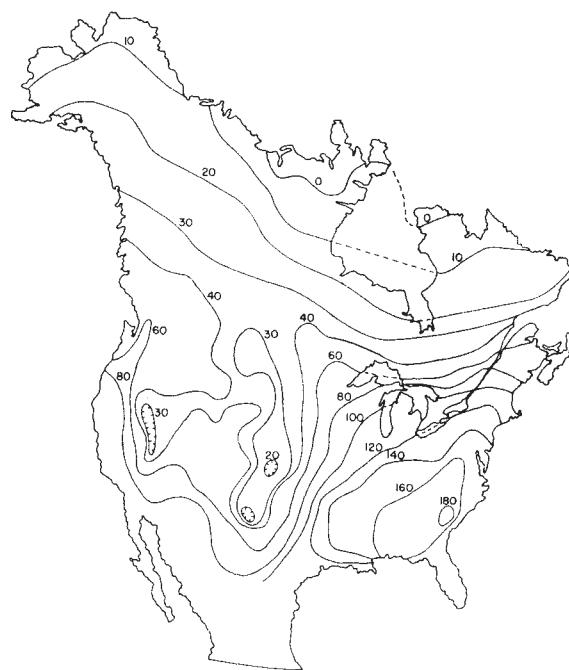


Fig. 1 Tree species richness in Canada and the United States. Contours connect points with the same approximate number of species per quadrat.

south of 50° N, and $2\frac{1}{2}^\circ \times 5^\circ$ (longitude) north of 50° N. Range maps¹³⁻¹⁵ of the 620 indigenous tree species (that is any ligneous plant growing to ≥ 3 m anywhere in its range¹³) were juxtaposed, and the number of species occurring in each quadrat was counted. Physical and climatic attributes of each quadrat (listed in Table 1) were obtained from published maps¹⁶⁻²². The effect of varying quadrat area was controlled statistically.

Figure 1 shows the spatial distribution of tree species richness in North America. Contours were drawn by eye; no exceptional quadrats fall within the indicated contours. A pronounced latitudinal gradient is apparent only in the east. Maximum richness occurs on the high plateau south of the Appalachian mountains, whereas striking minima are found immediately east of the Rocky and Sierra Nevada mountains. Peninsulas do not have notably fewer species. This pattern contrasts with those observed for birds and mammals, where maxima are found in the Rockies and Appalachians, local minima on peninsulas⁵⁻⁷, and fewer species in the southeast.

What accounts for these patterns? Species richness is correlated with many of the factors examined (Table 1), but most strongly with realized annual evapotranspiration (all water evaporated from the soil or transpired from vegetation). The relationship is non-linear; a logistic model, fitted by least-squares non-linear regression²³, adequately describes the data (Fig. 2):

$$\text{TSR} = 185.8 / [1.0 + \exp(3.09 - 0.00432 \text{ ARET})]$$

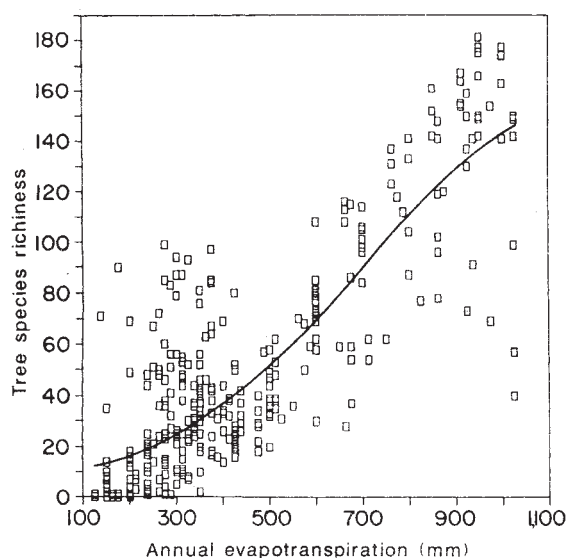
where TSR is tree species richness and ARET is annual realized evapotranspiration in mm ($F = 758.2$, $P < 0.0001$, $r^2 = 0.762$). Multiple regression showed that the residual variation is most closely related to two factors: diversity is greater in quadrats where the range of elevation is great, and it is less on the coast, especially at low latitude. Both stepwise and backward multiple regressions explain 2% more of the variation through other climatic factors (though not entirely the same set), such that $R^2 = 0.86$. It is notable that, after accounting for climate and topography, no significant variation was related to quadrat area, seasonal climatic variability, or the facts that an area had been glaciated or is on a peninsula (entered as dummy variables). A preliminary analysis of the diversity of terrestrial vertebrates yields very similar results (D.J.C., manuscript in preparation).

Table 1 Correlation between characteristics of the environment and tree species richness with and without controlling for evapotranspiration

Factor	Correlation with richness	
	<i>r</i>	<i>r</i> ₁
Latitude	-0.759***	-0.485***
Longitude	-0.378***	0.095*
Precipitation, mean annual	0.437***	NS
Precipitation, annual variation	0.109*	NS
Precipitation, variation within quadrat	NS	0.111*
Temperature, mean annual	0.770***	0.504***
Temperature, annual variation	-0.614***	0.420***
Evapotranspiration, mean annual	0.841***	NS
Evapotranspiration, variation within quadrat	0.133*	0.123*
Insolation, mean annual	0.567***	0.455***
Insolation, variation within quadrat	0.339***	0.276***
Quadrat area	NS	0.182***
Elevation, median	NS	0.484***
Elevation, variation within quadrat	NS	0.503***
Peninsula	NS	-0.177***
Coast	-0.229***	-0.308***
Coast/latitude	NS	-0.273***
Glaciated	-0.431***	NS

NS, not significant; *, $P < 0.05$; ***, $P < 0.001$. Correlations shown are simple linear Pearson correlation (with species (*r*)) and partial correlation with species richness after controlling for evapotranspiration (*r*₁).

Fig. 2 Tree species richness as a function of total annual realized evapotranspiration ($n = 366$). Solid line, fitted logistic model.



Why is evapotranspiration preminent? It is not simply a surrogate for latitude, because evapotranspiration explains significantly more of the variation in species richness ($t = 4.53$, $P < 0.001$). Evapotranspiration is highly correlated with terrestrial primary productivity^{24,25} and is thus a measure of community energy use. This observation is most consistent with the hypothesis that energy is partitioned among species such that the total available energy limits species richness³⁻⁴. Other factors induce variability around the limits determined by energy: for example, physically complex environments, like mountains, may favour more even energy partitioning among species, and thus permit relatively more species to occur together.

Perhaps our most interesting result is that 86% of the large-scale variability in tree diversity in North America can be statistically explained as a function of average climatic conditions and topography, with no reference to historical factors (glaciation and dispersal). In contrast, it is generally accepted that British and European flora are reduced in richness relative to the American flora due to barriers to post-glacial recolonization in

Europe^{9,26}. We tested this supposition by examining tree species richness in $2\frac{1}{2}^\circ \times 5^\circ$ quadrats in Great Britain and Ireland^{27,28}. If historical factors are critical, richness should be lower in Britain than in America. However, the observed number of species in the British Isles is very close to the number predicted from the North American regressions (Table 2; note that in Fig. 2 observations vary by about ± 15 species from the predicted value). Other recent studies similarly implicate the predominant influence of contemporary climate on species richness^{4,29}. Our analyses cannot unequivocally exclude the influence of historical factors; however, the most parsimonious interpretation of our results appears to be that species richness in trees varies among regions, both in North America and the British Isles, principally as a function of varying amounts of energy available to the community.

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Table 2 Prediction of the number of tree species occurring in Great Britain and Ireland from regressions on North American data

Quadrat	Predicted from		Observed
	Simple regression on evapotranspiration	Multiple regression on several factors	
Ireland			
33°-35.5° N			
6°-11° W	45	37	42
30.5°-33° N			
6°-11° W	45	30	41
Great Britain			
57.5°-60° N			
1°-6° W	45	37	36
55°-57.5° N			
1°-6° W	45	36	40
52.5°-55° N			
1°-6° W	52	31	49
52.5°-55° N			
1° W-1.5° E	45	42	46
50°-52.5° N			
1°-6° W	52	29	50
50°-52.5° N			
1° W-1.5° E	52	45	48

Predictions are for $2.5^\circ \times 5^\circ$ quadrats, and are based on simple regression on annual realized evapotranspiration; or on multiple regression on evapotranspiration and other factors (see text).

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Reproductive and genetic consequences of founding isolated lion populations

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Species survival is critically dependent on reproductive performance, a complex physiological process under rigorous genetic control. Classical studies of inbreeding in laboratory animals and livestock have shown that increased homozygosity can adversely affect spermatogenesis, ovulation and perinatal mortality and morbidity¹⁻³. For wild populations, the consequences of inbreeding depression have not been examined intensively, although our recent studies of the African cheetah revealed a striking degree of genetic uniformity^{4,5} combined with an extremely high incidence of structurally abnormal spermatozoa (>70%) in captive⁶ as well as free-ranging⁷ males. In this study, we report definitive evidence that the reproductive function of free-ranging mammals can be impaired as a result of demographic contraction followed by inbreeding. In an examination of three distinct lion populations (two from the Serengeti ecosystem in East Africa and a third descended from lions in the Gir Forest of western India), a direct correlation was observed between genetic variability and two physiological traits, incidence of abnormal sperm and circulating testosterone, a critical hormone for spermatogenesis.

The lion (*Panthera leo*) is an attractive candidate for studying the interrelationship of genetics and reproduction. Lions have been studied extensively for centuries, and their numbers in Africa are vast (estimated as high as 200,000)⁸. The demography and behaviour of the lions in the Serengeti ecosystem (*P. l. leo*)

and the Gir Forest Sanctuary (*P. l. persica*) have been monitored for 20 years or more⁹⁻¹⁵.

The lion is the most social of all the Felidae. Pride composition may remain more or less stable for several years and is affected primarily by birth, death, emigration of subadults and takeovers by non-resident coalitions of males^{12,13}. The pride core is a closed society of lionesses, all genetically interrelated. Inbreeding is normally avoided because, with rare exceptions, young males abandon the pride by 3.5 years of age, reducing the potential for incestuous matings¹³. Additionally, adult lionesses solicit matings from nomadic males or males from neighbouring prides, making inbreeding extremely infrequent. A possible exception is in severely restricted habitats, where a lack of unrelated partners inevitably results in incestuous sexual encounters.

Three distinct lion populations were surveyed, two in the Serengeti ecosystem in Tanzania, East Africa: one in the Serengeti National Park and the second in the Ngorongoro Crater. Approximately 3,000 lions inhabit the Serengeti Plains, an area of >25,000 km². The Ngorongoro Crater is a large volcanic caldera in the southeastern portion of the Serengeti ecosystem containing one of the highest lion population densities (100 animals in 260 km²) in Africa¹³. Plentiful prey biomass and a suboptimal habitat immediately outside the Crater provide little incentive for lions to leave the area. Occasionally, resident lions emigrate but no immigration into the Crater has been recorded since 1975 when continuous identification of individual lions began¹³. Thus, the Ngorongoro Crater is essentially a self-contained ecological niche containing a distinct and isolated population of lions. The present population is known to have descended from a small number (6-15) of founders which survived a 1962 epizootic of biting flies (*Stomoxys calcitrans*)¹⁶. The population gradually increased to its present size in 1975 and has remained stable since^{12,13}. The third group surveyed originated from lion stock of the Gir Forest Sanctuary located in the Gujarat Province of western India. These Asian lions, which show several morphological distinctions from their African counterparts^{14,15}, are a relict group of <250 individuals which experienced a severe population contraction (to <20 animals) in the first quarter of this century¹⁷. The lions sampled were in captivity at the Sakkarbaug Zoo near the Gir Forest.

In parallel studies, blood samples from lions of each popula-

Fig. 1 Pleiomorphic sperm forms in the ejaculates of lions. Males were anaesthetized with Telazol (Tiletamine plus Zolazepam, Warner Lambert, 3 mg per kg) administered with a dart gun. Semen was obtained by a standardized electroejaculation procedure^{6,7}. After fixing seminal aliquots from each animal in 1% glutaraldehyde, the morphologies of 900 individual spermatozoa from each male were assessed by phase contrast microscopy (×400). *a*, Normal; *b*, tightly coiled flagellum; *c*, missing mitochondrial sheath; *d*, abnormal acrosome and deranged midpiece; *e*, macrocephalic with abnormal acrosome; *f*, microcephalic with missing mitochondrial sheath; *g*, bent flagellum; *h*, bent neck with residual cytoplasmic droplet.

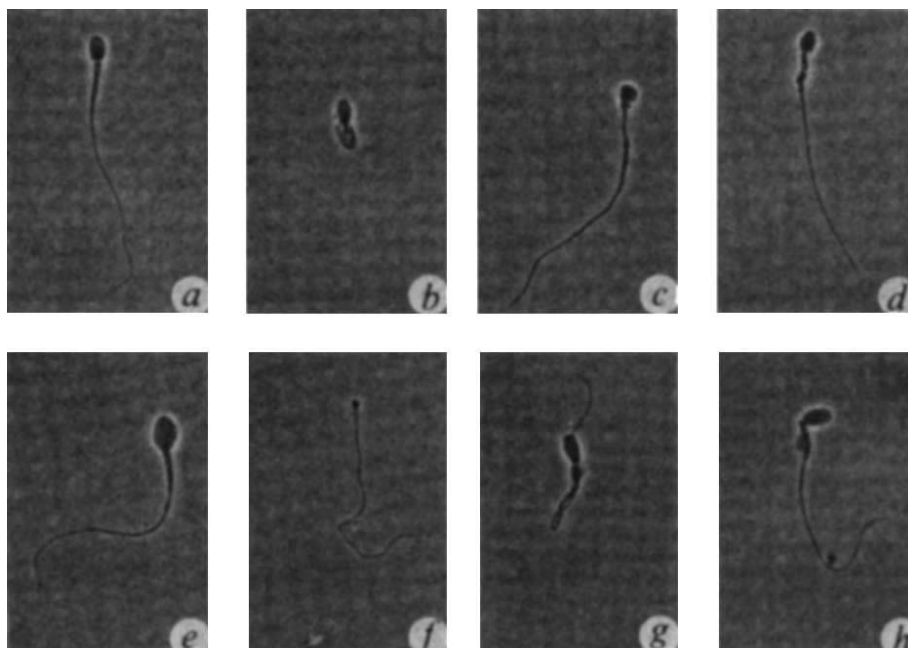


Table 1 Ejaculate characteristics of lions from the Serengeti Plains, the Ngorongoro Crater and the Sakkarbaug Zoo

	Serengeti National Park <i>n</i> = 8	Ngorongoro Crater <i>n</i> = 9	Sakkarbaug Zoo <i>n</i> = 8
Lions tested			
Ejaculate volume (ml)	9.4 ± 1.4 ^a	8.5 ± 0.8 ^a	5.9 ± 0.7 ^b
Spermatozoal motility (%)	91.0 ± 4.2 ^a	83.0 ± 4.6 ^a	61.0 ± 3.7 ^b
Sperm per ejaculate (×10 ⁶)	34.4 ± 12.8	25.8 ± 11.0	13.3 ± 2.8
Motile sperm per ejaculate (×10 ⁶)	228.5 ± 65.5 ^a	236.0 ± 93.0 ^a	45.3 ± 9.9 ^b
Total sperm abnormalities (%)	24.8 ± 4.0 ^a	50.5 ± 6.8 ^b	66.2 ± 3.6 ^b
Type:			
(1) Macrocephalic	0.6 ± 0.2	0.3 ± 0.1	0.0 ± 0.0
(2) Microcephalic	0.2 ± 0.04	0.2 ± 0.06	0.1 ± 0.05
(3) Biflagellate	0.04 ± 0.03	0.03 ± 0.02	0.0 ± 0.0
(4) Bicephalic	0.2 ± 0.04	0.8 ± 0.6	0.4 ± 0.2
(5) Abnormal acrosome	1.1 ± 0.3 ^a	0.9 ± 0.1 ^a	3.6 ± 0.7 ^b
(6) Abnormal midpiece	1.9 ± 0.4 ^a	3.7 ± 1.0 ^a	0.6 ± 0.2 ^b
(7) Tightly coiled flagellum	2.3 ± 0.5 ^a	8.5 ± 3.3 ^b	13.7 ± 2.4 ^c
(8) Detached head	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a	6.6 ± 1.8 ^b
(9) Bent midpiece with droplet	2.3 ± 0.6 ^a	12.4 ± 3.0 ^b	5.2 ± 1.0 ^c
(10) Bent midpiece	2.1 ± 0.6 ^a	4.2 ± 1.0 ^{ab}	5.0 ± 0.8 ^b
(11) Cytoplasmic droplet	12.5 ± 2.2	17.2 ± 2.9	16.9 ± 3.3
(12) Bent flagellum	0.9 ± 0.3 ^a	1.2 ± 0.5 ^a	11.3 ± 2.1 ^b
(13) Bent neck	0.7 ± 0.1	1.1 ± 0.2	2.8 ± 1.1

Each ejaculate was collected in a warmed (37 °C) plastic container. Sperm motility and concentration values were evaluated immediately in the field^{6,7} using a phase-contrast microscope powered by a portable generator. These values as well as semen volume were used to provide the index of motile sperm per ejaculate. Fig. 1 legend describes general methodology for morphological assessments. Within each row, values with different superscripts are significantly different ($P < 0.05$); values within rows with no superscripts are similar ($P > 0.05$).

tion were separated into erythrocytes, leucocytes and plasma for preparation of isozyme extracts¹⁸. An electrophoretic survey of 46–50 allozyme loci revealed measurable differences in the extent of genetic variation among the three populations. The Serengeti group ($n = 27$) had abundant genetic variation with the proportion of the loci polymorphic, $P = 10.9\%$. The mean heterozygosity (H , the average frequency of heterozygous loci in one individual) was 0.038. The Crater lions ($n = 19$) expressed less variability ($P = 4.3\%$; $H = 0.014$), and there was allelic variation at a subset of the loci that were polymorphic in the surrounding (and presumably founder) population of the Serengeti Plains. The Asiatic lion samples ($n = 28$) from the Sakkarbaug Zoo were unique among the three groups in demonstrating complete genetic monomorphism at each of the typed loci.

To quantify physiological differences among lions from the three environments, seminal and endocrine traits were evaluated and related to genetic findings. Semen samples were collected from anaesthetized lions in July and August in Africa and November in India using a standardized electroejaculation procedure^{6,7}. Based on previous observational studies, the precise age of each African animal was known and none of the males had been observed copulating or consorting with oestrous females during the 6 weeks before semen collection. The age (Serengeti, 5.3 ± 0.8 years; Crater, 4.5 ± 0.5 years; mean $\pm$ s.e.m.) and age range (Serengeti, 2.5–7.0 years; Crater, 3.0–7.5 years) of each African group conformed to sexual maturity, onset of spermatogenesis normally occurring at 24–36 months (ref. 9). An accurate average age could not be calculated for the Asian lions because one was a founder of unknown age caught in the wild; however, the age range of the captive-born descendents (4.5–9.0 years) was similar to that of the African groups. Before, during and after the electroejaculation interval, blood samples were collected and sera were stored and later analysed for luteinizing hormone (LH) and testosterone concentrations.

Compared to African lions, the Asiatic lions showed depressed mean ejaculate volume, a sperm motility rating and fewer motile sperm per ejaculate (Table 1). Although the two African populations were comparable in these traits, 50.5% of the spermatozoa from the Crater lions were morphologically abnormal, a level twofold greater than that detected in the Serengeti lions (Table 1). Lions at the Sakkarbaug Zoo averaged

16% more pleiomorphic spermatozoa than the Crater group and a threefold greater incidence of morphological abnormalities than observed in the Serengeti population (Table 1). The greatest increases in structural defects were observed in the number of spermatozoa with a tightly coiled flagellum or deranged midpiece (Table 1; Fig. 1). Between the Crater and Sakkarbaug Zoo groups, the specific proportion of abnormalities frequently varied, with the former producing more sperm with midpiece defects whereas sperm from the latter generally were affected with more acrosomal and flagellar deformities. Certain Crater and Sakkarbaug Zoo lions were predisposed to a high incidence of very unusual abnormalities. For example, 5.4% of all sperm cells in one Crater male were afflicted by bi- or tricephaly whereas in two Asian males, 12.4% and 15.4% of all sperm heads were decapitated from the flagellar portion of the cell. The precise aetiology of specific pleiomorphic forms is controversial¹⁹. However, defect types 1–8 (Table 1) are generally associated with spermatogenic dysfunction whereas type 9–13 defects originate as sperm migrate through the excurrent duct system^{6,7}. In either case, the fertilization potential of an abnormal spermatozoon is probably impaired because of severely reduced cell motility, acrosomal integrity and perhaps even altered DNA content in instances of micro- or macrocephaly²⁰.

LH is an important gonadotropin in the male and is primarily responsible for stimulating testosterone production by the Leydig cells. As measured eight times over an ~80 min interval, the LH profile was similar in the three populations (Fig. 2). Mean testosterone concentrations, however, were about threefold less ($P < 0.05$) in the Crater and Sakkarbaug Zoo populations compared to the Serengeti group (Fig. 2). The overall temporal testosterone profile was not different ($P > 0.05$) in Crater compared to Sakkarbaug Zoo lions; however, as the blood sampling interval progressed beyond 20 min, testosterone concentrations were two- to fourfold less in Sakkarbaug Zoo compared to Crater lions.

The integration of pituitary and gonadal hormones is essential for normal spermatogenesis^{21,22}. African lions in the restricted Ngorongoro habitat or descended from the remnant Gir Forest population had lower testosterone levels even when basal concentrations of the controlling hormone LH were equivalent. Reduced testosterone secretion can contribute to impaired

spermatogenesis by interfering with meiotic events associated with normal gamete formation^{23,24}. The physiological basis for lowered testosterone production is unclear, but could involve impaired hypothalamic, pituitary or testicular function.

Overall, these results can be interpreted in one of two ways. It is possible that the differences among populations (particularly between the African and Asian groups) was, in part, attributable to variations in environmental factors (seasonality, nutrition, pride dynamics, copulatory activity, or whether the lions were free-ranging or captive-bred). These variables were difficult to control in the present study but nonetheless were minimized as a result of our previous efforts involving longitudinal ethological observations of both African and Asian populations. Environmental variability is an unlikely explanation of the data for several reasons. First, lion reproductive performance is not markedly seasonal⁹ and the times of semen evaluation corresponded to previous field observations of breeding activity in each population¹²⁻¹⁵. Secondly, although the Serengeti and Ngorongoro Crater populations are strikingly distinct in genetic and reproductive correlates, these lions reside in adjacent ecosystems involving the same climate, pride structure and prey base. Further, these two groups, sampled at the same time of year, were age-matched and precisely selected on the basis of inter- and intra-pride reproductive experience. Finally, comparing reproductive characteristics between the free-living African and captive Asian populations seems justifiable because there is at least one precedent for male reproductive traits not varying between captive and free-living felids; there was no difference in semen characteristics and circulating testosterone levels between cheetahs maintained in various US zoological parks and those free-ranging in the Serengeti ecosystem⁷.

A more logical explanation of the results is that as allozyme variation decreased among geographical groups, ejaculate quality declined, with sperm structure appearing most vulnerable. Spermatozoal morphology is not influenced by ejaculatory abstinence in a variety of felids (including domestic cats, cheetahs⁶ and clouded leopards²⁵) but rather remains consistent even among seminal aliquots collected as frequently as three times per week. Additionally, the semen characteristics measured in this study were based on a highly standardized electroejaculation protocol (all males received the same number and type of stimuli under the same level of surgical anaesthesia) previously shown to be effective in discriminating among male felids with variable reproductive performance records²⁵. Furthermore, not only was there an inverse correlation between genetic variability and spermatozoal integrity, but there was a correlative and significant depression in circulating testosterone in both the lion populations with lower quality spermatozoa. Taken together, these results implicate the genetic history of the populations in contributing to reproductive status in a manner reminiscent of reproductive impairment in deliberately inbred mice and farm livestock¹⁻³. The data from the two African lion populations suggest that a restricted natural habitat combined with a well-defined population bottleneck affected genotype and spermatozoal integrity. The Asian lion data strongly support the hypothesis that diminished genotypic diversity adversely influences seminal traits as these lions produce ejaculates with more pleiomorphic sperm forms and fewer motile spermatozoa than even the reproductively compromised Crater lions.

Overall reproductive success in lions could be influenced by altered degrees of spermatogenesis and testosterone-influenced sexual activity in concert with normal inter- and intra-pride dynamics. The ultimate effect of impaired reproductive function in the Crater lions may not be recognized for years, if ever. However, recent breeding performance of some of the Sakkarbaug Zoo lions may warrant concern. One of the 6.5-year-old test males mated to oestrous females has failed to produce offspring and three of the other males have produced only single-cub litters or stillborn cubs in the past two years. Also of

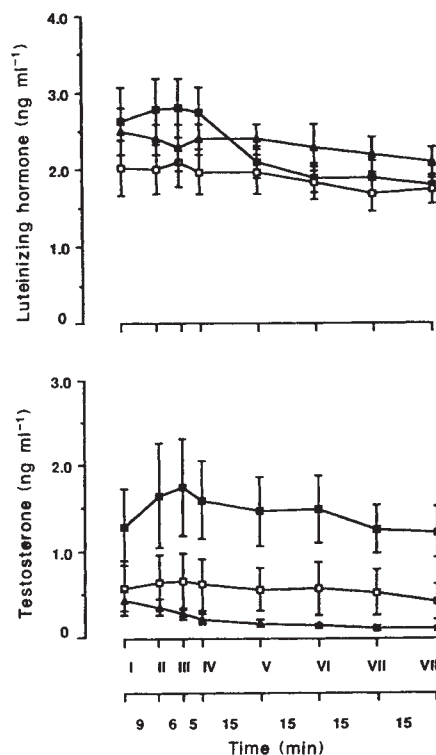


Fig. 2 Mean ($\pm$ s.e.m.) concentrations of serum LH and testosterone in lions from the Serengeti National Park (■), the Ngorongoro Crater (□) and the Sakkarbaug Zoo (▲). Roman numerals indicate time of blood sampling: I, post-anaesthesia, pre-electroejaculation; II-IV, end of electroejaculation series 1, 2 and 3, respectively; V-VIII, 15, 30, 45 and 60 min after electroejaculation. Serum samples were analysed using validated radioimmunoassays^{7,25,27,28}. A biomedical computer program (Statistical Analysis System Institute) was used to evaluate differences in repeated measures of LH and testosterone.

significance is the potential influence of a homozygous genotype on the health status of the species, as illustrated in the devastating susceptibility of the cheetah to a corona virus, feline infectious peritonitis²⁶.

Together, these results demonstrate the conceivable impact of a known population bottleneck on physiological function of a wild mammalian species. Such findings should be of particular relevance in emphasizing the need for skilful management of captive wildlife collections, where restricted population size is the norm rather than the exception.

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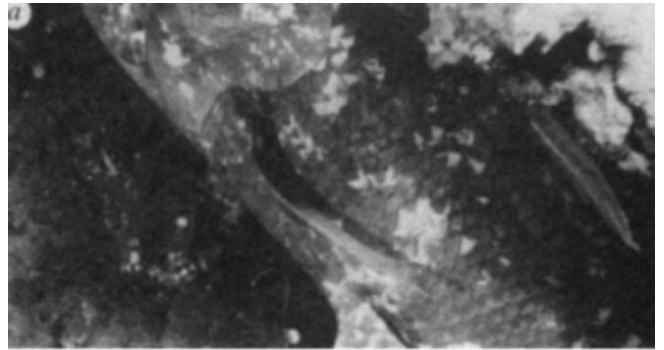


Fig. 1 Contact with the bottom *a*, with pectoral fins folded laterally or *b*, with tips of both paired fins touching the bottom. *c*, Drifting in an upwelling current with paired fins functioning as hydrofoils: they stabilize and correct the 'underwater flying'.

Locomotion of the coelacanth *Latimeria chalumnae* in its natural environment

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The coelacanth *Latimeria chalumnae* is the only living relic of a fossil group of crossopterygian lobe-finned fish^{1–4}. We describe observations of its locomotion in a natural environment using six individuals observed from the research submersible *Geo* in the Indian Ocean at a depth of between 117 and 198 m. Past speculation on the pattern of locomotion has included crawling with the paired fins on the rocky ocean bottom, stalking like a large piscivorous grouper, or even fast swimming in open water^{4–6}. Our observations show *L. chalumnae* to be a nocturnal piscivorous drift-hunter, moving very slowly in up- or downwelling currents, while paired fins stabilize and correct the drift motion. Paired and unpaired lobed fins are able to generate thrust. Fast starts are performed with the large caudal fin. Paired fins were not used for locomotion along the bottom. They alternate synchronously in a pattern common in tetrapod locomotion.

L. chalumnae is found near the remote volcanic islands of the Comores in the Western Indian Ocean at a depth of ~200 m. Since their discovery in 1938 no observations of free-ranging fish in their natural environment have been made, although locomotory patterns have been observed on line-fished individuals taken to the surface and released in shallow water (refs 7–9, J. L. Geraud, personal communication). These studies indicated a great flexibility of the second dorsal and anal fins which produced some forward thrust by sculling action. The paired fins did not produce swimming movements and the caudal fin did not move at all. The fish remained neutrally buoyant. Line-caught coelacanths are probably under extreme metabolic stress and might suffer decompression sickness after fast surfacing.

We performed 40 dives at 30 different locations around the entire coast of Grande Comore and the north coast of Anjouan and found six fish of ~120–180 cm body length off a 2-km stretch of the coastline of Grande Comore. These were observed for a

total of 496 min at night only, between 21:00 and 03:30, and we have used more than 300 photographs, 90 min of 16-min cine film and 30 min of videotape recording for cinematographic analysis. The fish seemed undisturbed by the submersible or by the lights. Two of the six individuals were sitting on the bottom and four were in slow motion. During contact with the bottom, the paired fins were not used for any kind of locomotion. This was confirmed by a six-hour observation of a single individual. It frequently touched the bottom, either with the belly alone, keeping the pectoral fins laterally folded (Fig. 1*a*), or with the belly and the lobes of the paired fins together (Fig. 1*b*); no locomotion was observed.

All individuals took advantage of up- or downwellings and drifted slowly with the current. Both paired fins stabilized and corrected the 'underwater flying' used in a bird wing fashion (Fig. 1*c*). During drifting, all individuals seemed perfectly oriented in that they avoided obstacles in their environment, apparently detecting them well in advance. Although no feeding

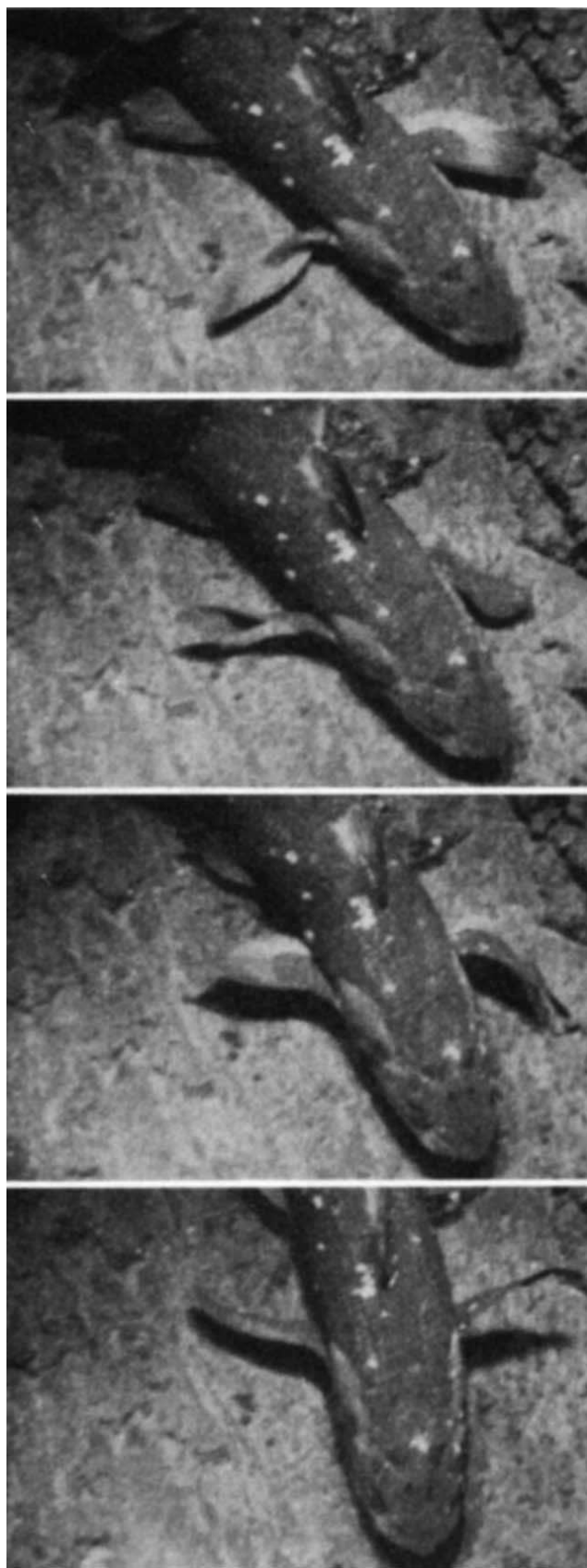


Fig. 2 Sequences of pectoral movements during a right turn. At the beginning, the left pectoral performs a strong downward stroke while at the same time the right pectoral fin rotates 180° around its main axis, causing a braking effect. Note the left pectoral fin changes its angle of thrust during upstroke.

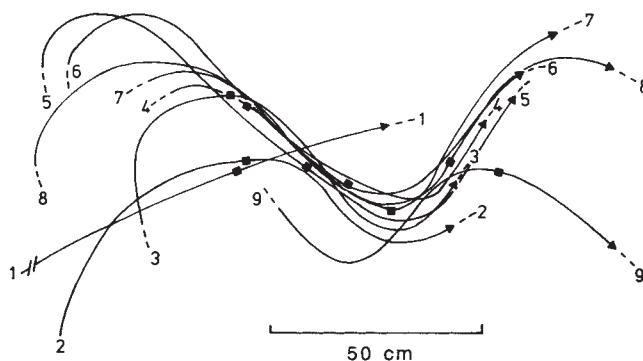


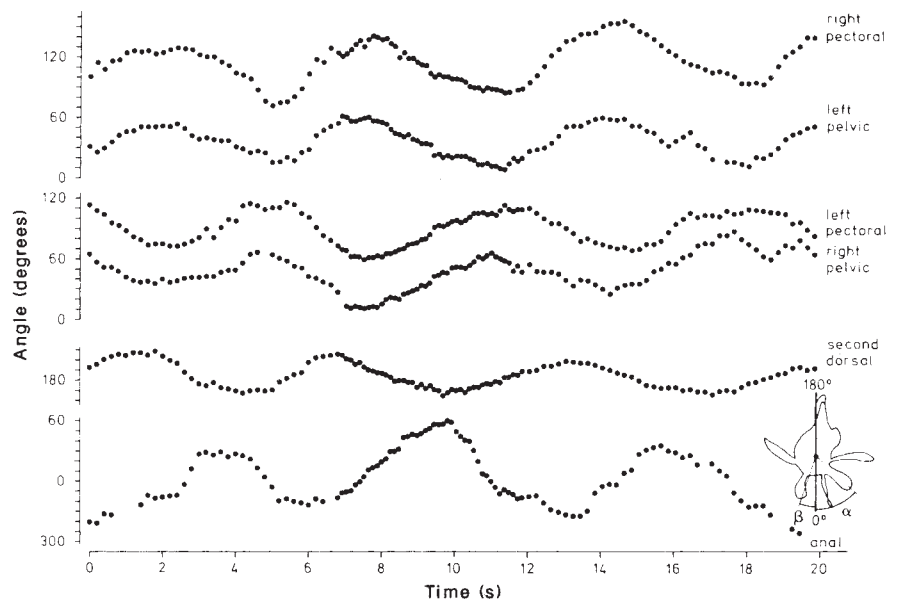
Fig. 3 Fast start sequence of a specimen estimated to be 1.5 m in total length and filmed at 25 frames per s. Lines indicate the main body axis, dashed lines unsharp contours, and the arrows point in the direction of the head. The assumed centre of mass (■) lies behind the posterior edge of the erected first dorsal, the length of which was 0.14 of the total body length, calculated from exact side views of the same animal. Preparatory displacement stage (stage 2) lasted 0.04 s; the forward propulsion stage lasted 0.2 s and consisted of the preacceleration stage (3), acceleration stage (4–6) and postacceleration stage (7). Mean acceleration a_Φ can be obtained from the velocity difference between stages 7 ($v = 4.4 \text{ m s}^{-1}$) and 2 ($v = 0.7 \text{ m s}^{-1}$), divided by the duration from mid-stage 2 to mid-stage 3–7 (0.16 s) to give $a_\Phi = 23.1 \text{ m s}^{-2}$. Perspective errors and errors in estimation of absolute body length are possible.

was observed, the coelacanth migrated by drifting into shallower water at $\sim 120 \text{ m}$ depth where prey fish were more abundant (a full account of the coelacanth's habitat is in preparation). All individuals irregularly performed a curious head-stand lasting for up to 2 min, the purpose of which is unknown. Furthermore, the body can be held steadily in any position; two were observed drifting with the belly facing the surface and also swimming backwards. During substrate contact or during drifting, the small epicaudal lobe of the large caudal fin can be moved up to $\pm 90^\circ$ laterally to the main body axis. Although not experimentally proven, the coelacanths seemed able to detect direction and speed of the current. Their behaviour also appeared to indicate that they possess powers of electropception, probably with the help of the rostral organ¹⁰. The drift movement was frequently interrupted by active swimming motions when the fish either changed direction or oriented itself to the bottom, using the pectoral and the unpaired lobed fins to create thrust. The pectoral fins performed a dorso-ventral up and down motion. During downstroke they are positioned obliquely against the direction of motion, but during upstroke the angle of attack is reversed. The paired fins are used as a hydrofoil. Figure 2 shows a right body turn where details of thrust-generating movements of the left pectoral are visible. Swimming in an arc was assisted by the large expanded lobes of the caudal fin, which turned with the wave of curvature.

Four fast starts were observed: the body performed an S-shaped undulation with the caudal fin generating the main thrust (Fig. 3). Acceleration during one fast start, lasting 0.24 s, was calculated. The mean acceleration was $\sim 2.4g$, which can be compared with the value for a pike of $1.1g$ and for a trout of $4.9g$ (refs 11, 12). Each pair of lobed fins, and the unpaired fins, can move at various stroke frequencies, which increase with swimming speed. During slow forward motion, paired fins had an identical stroke frequency, with unpaired fins slightly faster. During fast motion the frequencies of the pectoral and pelvic fins were again similar, but the unpaired fins were slower.

Figure 4 shows fin coordination and phase relationships during slow forward motion. The pectoral fins were synchronized

Fig. 4 Fin coordination during slow forward motion. The movements of the fins were measured as angles, the fish being filmed from behind with all fins visible (see diagram) taking the angle between the fin tip and a vertical line through the centre of mass (see Fig. 3 symbol, ■). Angles: α , fin angles of right body side counting counterclockwise; β , fin angles of left body side counting clockwise; β , for unpaired median fins. Time in seconds is measured after the start of movement. The data points between 7–12 s are more dense because this range was at first analysed from every third frame, but later every fifth frame was used.



with a phase difference Φ of approximately half a cycle (180° ; ~ 3.8 s); the same relationship held for the pelvic fins. The left pectoral and right pelvic, and the right pectoral and left pelvic fins were synchronized. The synchronization was stable for the entire film sequence (more than 2 min). During fast forward movements, however, phase differences increased between different fin types, so that alternating coordination between opposite, paired fins became less precise.

Our time-limited observations confirm that all paired and unpaired lobed fins of *L. chalumnae* are adapted for slow swimming. They are able to produce forward thrust. Paired fins are not used for locomotion on the bottom, such as crawling or stalking. Thus the locomotory pattern of the coelacanth does not confirm early predictions^{5,6}. Yet the alternating coordination of the paired fins is a pattern common in tetrapods and a few bottom-living fish^{13,14}, including lung-fish (H. Greenwood, personal communication). The function of this neuromuscular coordination for alternating fin movements has yet to be demonstrated. Probably alternating fin movements achieve a better stabilization of the heavy, cigar-shaped body. Such coordination could indicate another preadaptation in the crossopterygian group that could have facilitated the transition to locomotion on land^{15–18}.

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α_1 -Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle

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Receptor-mediated increases in intracellular Ca^{2+} levels can be caused by release from intracellular organelles and/or influx from the extracellular fluid. Noradrenaline (NA) released from sympathetic nerves acts on α_1 -adrenoceptors to increase cytosolic Ca^{2+} and promote smooth muscle contraction¹. In many cells activation of α_1 -adrenoceptors causes formation of inositol 1,4,5-trisphosphate which promotes Ca^{2+} release from intracellular stores^{2,3}. The mechanism by which receptor activation opens cell surface Ca^{2+} channels is not known, although in some cases it may be secondary to formation of inositol phosphates^{4,5} or release of stored intracellular Ca^{2+} (ref. 3). However α_1 -adrenoceptors have recently been shown to have different pharmacological properties in different tissues^{6–9}, and it has been proposed that different α_1 -adrenoceptor subtypes may control mobilization of intracellular Ca^{2+} and gating of extracellular Ca^{2+} influx^{7,9,10–12}. We here report evidence for two subtypes of α_1 -adrenoceptors which cause contractile responses through different molecular mechanisms. One subtype stimulates inositol phosphate (InsP) formation and causes contractions which are independent of extracellular Ca^{2+} , and the other does not stimulate inositol phosphate formation and causes contractions which require the influx of extracellular Ca^{2+} through dihydropyridine-sensitive channels. These results suggest that neurotransmitters and hormones may control Ca^{2+} release from intracellular stores and influx through voltage-gated membrane channels through distinct receptor subtypes.

In our previous studies¹³, we found that the irreversible alkylating agent chloroethylclonidine¹⁴ inactivated only a subpopula-

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tion of the α_1 -adrenoceptor binding sites labelled with radioiodinated BE 2254¹⁵ (¹²⁵I-BE) in different tissues. The binding sites in liver and spleen were almost completely inactivated by chlorethylclonidine while those in hippocampus and vas deferens were not affected¹⁶. To determine whether such differences were due to the existence of distinct receptor subtypes, we compared the potencies of competitive antagonists in inhibiting specific ¹²⁵I-BE binding in tissues where the sites are (liver, spleen) or are not (hippocampus, vas deferens) sensitive to inactivation by chlorethylclonidine. Figure 1 shows composite inhibition curves in liver and hippocampus for some of the drugs we tested.

BE 2254 (α_1 -selective) and yohimbine (α_2 -selective) were approximately equally potent in all four tissues. The Hill coefficients for these drugs were close to 1.0 (0.94 to 1.14), and inhibition curves were best fit by a one-site model. However, WB 4101 and benoxathian were more potent in hippocampus and vas deferens than in liver and spleen. The Hill coefficients were close to 1.0 in liver and spleen (0.95 to 1.07) but were significantly lower in hippocampus and vas deferens (0.63 to 0.82). The inhibition curves for these drugs were better fit by a two site model in hippocampus and vas deferens, but not in liver or spleen (Table 1). About half of the binding sites in hippocampus and vas deferens had affinities for WB 4101 and benoxathian similar to those in liver and spleen, while the others had a 10–20-fold higher affinity. Similar results have been reported previously for inhibition of ³H-prazosin binding by WB 4101 in hippocampus where the high and low affinity sites were termed α_{1a} and α_{1b} , respectively⁸. These results suggest that there are two subtypes of α_1 -adrenoceptor binding sites in hippocampus and vas deferens, but that liver and spleen contain mainly the low affinity (α_{1b}) subtype.

We wanted to determine whether these different binding sites were functional receptors. Schild plots for each antagonist in competitively inhibiting NA-stimulated contractions of vas deferens and spleen are shown in Fig. 2. Since the slopes were close to 1.0, pA_2 values calculated from these plots should equal the equilibrium binding constant for each antagonist at the

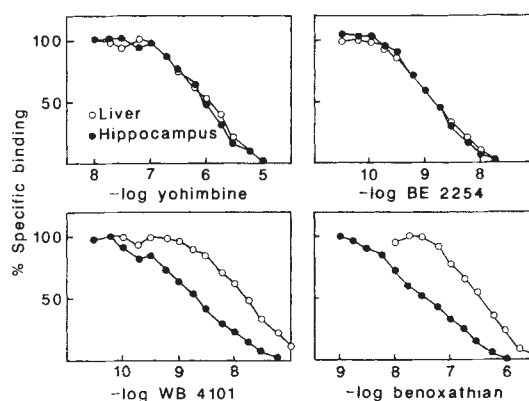


Fig. 1 Inhibition of specific ¹²⁵I-BE binding by competitive antagonists in rat liver and hippocampus. Binding was determined in a crude particulate fraction from rat hippocampus¹³ or in membranes purified from rat liver by Percoll gradient centrifugation²⁰. Briefly ¹²⁵I-BE (45–50 pM) was incubated with tissue (10–50 µg protein) for 20 min at 37 °C in 20 mM NaH₂PO₄/Na₂HPO₄ buffer (pH 7.6) containing 154 mM NaCl and drugs as indicated²¹. Reactions were terminated by dilution with 10 ml of 10 mM Tris-HCl buffer (pH 7.4) and filtration over glass fibre filters (Schleicher and Schuell no. 30) under vacuum. Filters were then washed with a further 10 ml buffer and radioactivity determined. Specific receptor binding was defined as binding displaced by 10 µM phentolamine. Data are plotted as % specific binding remaining in the presence of the indicated concentrations (M) of antagonist. Each value is the mean of data from three or four experiments performed in duplicate.

Table 1 Two-site analysis of inhibition of specific ¹²⁵I-BE binding by WB 4101 and benoxathian

	K_H	K_L	% R_L	P value
WB 4101				
Hippocampus	9.53	8.57	64	<0.05
Vas deferens	9.25	8.22	60	<0.05
Liver	—	8.08	100	—
Spleen	—	8.16	100	—
Benoxathian				
Hippocampus	9.04	8.18	46	<0.05
Vas deferens	8.96	8.09	60	<0.05
Liver	—	7.73	100	—
Spleen	—	8.13	100	—

Inhibition of specific ¹²⁵I-BE binding by each antagonist was determined in crude particulate preparations from rat hippocampus, vas deferens and spleen and in purified rat liver membranes as described in the legend to Fig. 1. Data from four to eight experiments performed in duplicate were combined and the best two-site fit determined by nonlinear regression analysis. Two-site models were compared with one-site models to determine whether the increase of goodness of fit was significantly more than would be expected on the basis of chance alone¹⁸ using a partial F -test. P values less than 0.05 were considered significant. IC_{50} values were converted to K_D values as described by Cheng and Prusoff¹⁹ to determine the K_D at the high affinity (K_H) and low affinity (K_L) binding sites. The proportion of binding sites in the low affinity form is denoted as % R_L . Competition curves in liver and spleen fit to only one site, which was designated K_L because of its similarity to this site in the other tissues.

functional receptor. The pA_2 values for yohimbine and BE 2254 were similar in vas deferens and spleen, and were similar to the K_D values determined in binding studies. However, both WB 4101 and benoxathian were 15–20-fold more potent in blocking NA-mediated contractions in vas deferens than in spleen. The pA_2 values in spleen and vas deferens were similar to the K_D values at the low and high affinity binding sites, respectively, suggesting that each binding site can serve as a functional receptor. Although vas deferens had approximately equal proportions of both types of binding sites, only the 'high' affinity (α_{1a}) subtype appeared to be involved in the contractile response.

Since stimulation of inositol phospholipid hydrolysis is thought to be the initial step in α_1 -adrenoceptor mediated responses^{2,3}, we wanted to determine if both receptor subtypes activated this system. K_i values for inhibition of NA-stimulated ³H-InsP accumulation by yohimbine and BE 2254 were similar in vas deferens and spleen, and similar to the pA_2 values determined against contraction in these tissues (data not shown). However, WB 4101 and benoxathian were 8–17-fold less potent in blocking NA-stimulated ³H-InsP formation than NA-stimulated contractions in vas deferens (Fig. 2). The pA_2 values for these drugs in blocking ³H-InsP formation more closely resembled those for blocking contraction of spleen, and were significantly ($P < 0.01$) different from those for blocking contraction of vas deferens. The $-\log K_i$ for WB 4101 in blocking norepinephrine-stimulated ³H-InsP formation in rat cerebral cortex (8.33 ± 0.10) and hippocampus (8.40 ± 0.13) also resembled the K_D value for the 'low affinity' α_{1b} subtype, demonstrating that only the α_{1b} subtype is linked to inositol phospholipid hydrolysis in these tissues.

These experiments suggested that the contractile response of rat vas deferens is mediated through an α_1 -adrenoceptor subtype (α_{1a}) which does not activate inositol phospholipid hydrolysis. Since release of inositol phosphates controls mobilization of intracellular Ca^{2+} , it was of interest to examine the importance of extracellular Ca^{2+} in the contractile responses. The Ca^{2+}

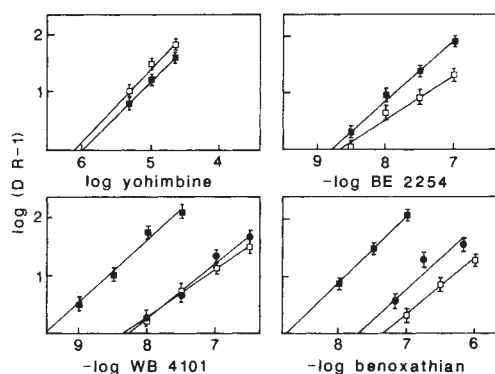


Fig. 2 Schild plots for competitive inhibition of noradrenaline-stimulated contractions and ^3H -InsP formation in rat vas deferens and spleen. ■, Contraction vas; □, contraction spleen; ●, ^3H -InsP vas. Noncumulative dose-response curves for noradrenaline-stimulated contractions of vas deferens were determined²¹ in the absence or presence of the indicated doses of antagonists. Spleens were hemisected²² and equilibrated under the same conditions for 90 min under a resting tension of 1 g, and primed with a dose-response curve to noradrenaline. After equilibration for a further 30 min, dose-response curves to noradrenaline were generated in a cumulative manner in the absence or presence of antagonists. Tissues were pre-equilibrated with each concentration of antagonist for 1 hr before dose-response curves were generated. Dose-response curves for noradrenaline induced increases in ^3H -InsP accumulation in 2 mm rings of vas deferens were determined as described by Fox *et al.*²³ in the presence of 0.1 mM pargyline and 0.1 mM pyrogallol in the absence or presence of antagonists. None of the antagonist concentrations caused significant reductions in maximum for any of the responses tested. The concentration of noradrenaline necessary to give a half-maximal response in the presence of each dose of antagonist was divided by the concentration giving a half-maximal response in the absence of antagonist to determine the dose-ratio (DR). Data are plotted by the method of Arunlakshana and Schild²⁴ as the $-\log$ antagonist concentration (M) vs the $\log$ (dose-ratio - 1). Each point is the mean $\pm$ s.e.m. of data from three or four experiments.

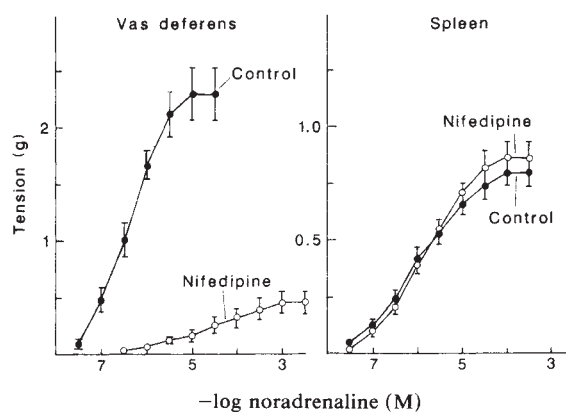


Fig. 3 Effect of nifedipine on noradrenaline-mediated contractions of vas deferens and spleen. Contractile responses were determined as described in the legend to Fig. 2. After equilibration and priming of the tissues, nifedipine ($1 \mu\text{M}$) was added to some preparations 12 min before beginning the dose-response curves. Each value is the mean $\pm$ s.e.m. of four determinations.

channel blocker nifedipine ($1 \mu\text{M}$) did not affect NA-stimulated contractions of spleen (Fig. 3) but markedly reduced NA-stimulated contractions of vas deferens in a manner consistent with antagonism of the receptor reserve¹⁷. Similar results were obtained when Ca^{2+} was removed from the bathing fluid and 0.1 mM EGTA was added, although the maximum contractile response was slightly reduced in spleen (data not shown). Since the contractile response of vas deferens was not completely abolished by nifedipine, it was possible that the small nifedipine insensitive component was mediated by the low affinity inositol phospholipid linked receptor subtype (α_{1b}) which also exists in this tissue. In the presence of nifedipine, 30 nM WB 4101 caused only a 6-fold shift to the right in the dose-response curve to NA in vas deferens although the same concentration caused more than a 100-fold shift in control preparations. The $-\log K_B$ for WB 4101 was calculated to be 8.24 in the presence of

nifedipine, similar to that at the low affinity (α_{1b}) binding site and the ^3H -InsP response.

Our results strongly support the existence of two pharmacologically distinct α_1 -adrenoceptor subtypes, both of which can mediate functional responses to NA. The α_{1b} subtype regulates inositol phospholipid hydrolysis and gives rise to contractions independent of extracellular Ca^{2+} , while the α_{1a} subtype appears to control the opening of dihydropyridine-sensitive membrane channels through an unknown mechanism to allow influx of extracellular Ca^{2+} . Although the contractions mediated by these two subtypes are absolutely distinguished by their requirement for extracellular Ca^{2+} in the tissues we studied, this will probably not hold true in all cases. Many cells utilize internal Ca^{2+} stores to provide transient initial signals, and require influx from the extracellular fluid for prolonged signals³. Ca^{2+} influx has been suggested to be activated directly by $\text{Ins}(1,4,5)\text{P}_3$, $\text{Ins}(1,3,4,5)\text{P}_4$ or by the Ca^{2+} released from intracellular stores³⁻⁵. However, the channels regulated by inositol phosphates and intracellular Ca^{2+} are generally insensitive to dihydropyridine channel blockers. The major difference in the mechanisms by which the different α_1 -adrenoceptor subtypes increases intracellular Ca^{2+} is probably not simply the involvement of extracellular Ca^{2+} , but rather in the control of influx through voltage-sensitive channels. α_{1a} -Adrenoceptors might control voltage-sensitive channels directly through a guanine nucleotide regulatory protein or indirectly through effects on ionic permeability, allowing depolarization and channel opening. Although the precise mechanism remains to be delineated, the existence of pharmacologically and functionally distinct α_1 -adrenoceptor subtypes will undoubtedly have important implications for our understanding of smooth muscle function.

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Differentiation potential of subsets of CD4⁺8⁺ thymocytes

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Precursor T cells in the thymus are contained within a subpopulation of thymocytes that lack the markers CD4 and CD8¹⁻⁵. We have examined the heterogeneity of these cells by flow cytometric analysis, and defined four subpopulations using the cell surface markers Thy-1, J11d^{6,7} and the IL-2 receptor (IL-2R). The J11d⁺ subset of CD4⁺8⁺ cells all bear the antigen Thy-1, and some express the IL-2R. Staining and RNA analysis of J11d⁺ cells suggest that some express receptors of the CD3 $\gamma\delta$ type, but none express CD3 $\alpha\beta$ receptors. In fetal thymus organ culture, the J11d⁺ cells diversify to form 'cortical type' CD4⁺8⁺ cells and 'medullary type' cells expressing either CD4 or CD8; *in vivo* they repopulate the thymus of an irradiated host and seed the periphery with T cells. In contrast, the J11d⁻ subset of CD4⁺8⁺ thymocytes do not all bear Thy-1 and none express the IL-2R, but some express antigen receptors of the CD3 $\alpha\beta$ type. They have more limited diversification potential in organ culture, and *in vivo* fail to recolonize the irradiated host in a homing-independent assay. We conclude that they are not precursor T cells, but rather a side-branch from the main line of T cell differentiation.

We studied the surface phenotypes of CD4⁺8⁺ thymocyte subpopulations in relation to the haematopoietic differentiation marker J11d because this marker distinguishes stages in the differentiation of both B and T lymphocytes. In particular, it is expressed on most thymocytes but not on peripheral T cells, and on primary but not memory B cells⁶. In the thymus, it distinguishes functionally incompetent (J11d⁺) from functional (J11d⁻) CD4⁺8⁺ cells⁷. Thymocytes of young adult BALB/c or C57BL/6 mice were treated with two cycles of anti-CD4 and anti-CD8, plus complement. Cells which survived this treatment (~1.5% of the starting population) were stained with fluorescein (FITC) conjugated anti-CD8 and phycoerythrin (PE) conjugated anti-CD4 to check the effectiveness of depletion, and for a variety of other surface markers.

Figure 1 shows the phenotype characteristics of the subsets of BALB/c CD4⁺8⁺ thymocytes defined by the marker J11d. In *a*, staining with J11d is displayed against low-angle light scatter (FSC), a parameter related to cell size. The largest 10% of cells, which have the greatest FSC, all bear J11d. In *b*, cells stained with J11d-FITC conjugate and a second layer reagent control are shown; *c* shows cells stained with anti-Thy-1 and J11d-FITC conjugate. In this sample, 87% of the cells were Thy-1⁺, and a subset of these (71% of the total) were J11d⁺. There were no Thy-1⁻, J11d⁺ cells. Cells stained with anti-IL-2R and J11d are shown in *d*. In this case, all the IL-2R⁺ cells (37% of the total) were contained in the J11d⁺ subset. The markers Thy-1, J11d and IL-2R therefore define four subpopulations of CD4⁺8⁺ thymocytes. There are cells which express none of the markers; cells which express only Thy-1; cells expressing Thy-1 and J11d; and cells which express Thy-1, J11d and IL-2R.

We examined the expression of T cell antigen receptor components on the J11d⁺ and J11d⁻ subsets of CD4⁺8⁺ cells by staining with antibodies against CD3 and T-cell receptor β -chain. Some J11d⁺ and some J11d⁻ cells expressed CD3, but the β -chain marker F23.1⁸ was confined to J11d⁻ cells (Fig. 2*a, b, c*). The surface expression of CD3 in the absence of F23.1 suggested that the J11d⁺ subset might include cells with CD3 $\gamma\delta$

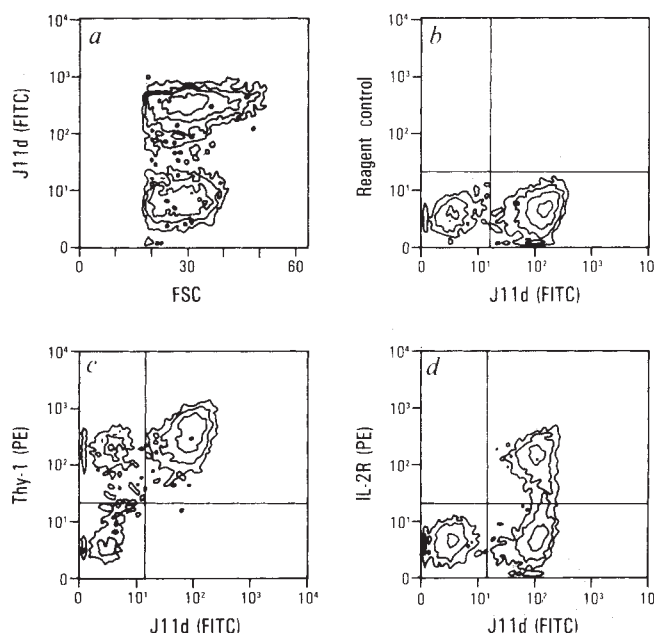


Fig. 1 BALB/c CD4⁺8⁺ thymocytes, stained to show the relationship between the marker J11d and other phenotypic characteristics. *a*, Cells stained with J11d-FITC conjugate, versus low-angle light scatter (FSC). *b*, Cells stained with T24 (anti-Thy-1)¹⁹ followed by PE-conjugated anti-mouse IgG (BioMeda), then 1 mg ml⁻¹ rat immunoglobulin (Cappel Labs, Cooper BioMedical) to block crossreactive antibody sites, then J11d-FITC. A reagent control, using HBSS plus 2% FCS instead of T24, is shown in *b*. Cells were similarly stained for IL-2R expression using PC61 (ref. 20) followed by PE anti-IgG, rat Ig, and J11d-FITC (*d*).

Methods. Thymocytes were washed twice in Hanks balanced salt solution (HBSS) containing 2% (v/v) fetal calf serum (FCS; Flow Labs), then resuspended in the same medium at $2-4 \times 10^7$ cells ml⁻¹. Antibodies to CD8 (3.168, anti-Lyt-2)¹⁷ and CD4 (RL172.4, anti-L3T4)¹⁸ were added to give predetermined optimum concentrations, and cells were left on ice for 45 min. Cells were pelleted by centrifugation and resuspended at $\leq 5 \times 10^7$ ml⁻¹ in guinea pig complement (Gibco). Two complete killing cycles were performed in sequence, to give highly pure double-negative cells. Viable cell yields, determined by Trypan blue exclusion, for BALB/c were $1.4 \pm 0.5\%$ (mean $\pm$ s.d. of 13 experiments). Aliquots of $0.1-1.0 \times 10^6$ cells were stained for flow-cytometric analysis. Optimum concentrations of staining reagents were determined in preliminary experiments. Cells were examined for residual CD4⁺ and/or CD8⁺ cells by two-colour direct immunofluorescent staining with anti-CD8-FITC and anti-CD4-PE (Becton Dickinson). Cells were analysed for one- or two-colour fluorescence using a FACS IV (Becton-Dickinson) with a single Argon 2W laser and logarithmic intensity scales. Intact cells were identified and dead cells excluded from analysis using a combination of low-angle and sideways light scatter. In most cases 10^4 events were scored, of which 70-90% were included in the analysis. In single-colour analysis, positive cells were defined by a marker set against cells stained with second-layer reagents only. In two-colour analysis using direct immunofluorescence, markers were set against the outermost contour of the contour plot generated by staining only with the other reagent. In 2-colour indirect immunofluorescence, controls involved substitution of diluent alone for either or both primary staining reagents.

receptors. To determine whether these cells also expressed the IL-2R, we stained CD4⁺8⁺ cells for IL-2R and F23.1 or CD3; all CD3 expression, as well as all F23.1, was on IL-2R⁻ cells (Fig. 2*d, e, f*). Surface staining therefore provided preliminary evidence that CD3 $\gamma\delta$ receptors were expressed mainly on J11d⁺ IL-2R⁻ cells, whereas CD3 $\alpha\beta$ receptors were found only on J11d⁻ cells.

Further evidence on the expression of the two categories of CD3-associated receptor was sought at the messenger RNA level (Fig. 3). Northern blots hybridized with a *C α* probe showed

Fig. 2 Two-colour flow cytometric analysis of the T-cell receptor markers F23.1 and CD3 against J11d and the IL-2 receptor on BALB/c CD4⁺CD8⁺ thymocytes. The F23.1⁺ cells were all J11d⁺ (b), but CD3⁺ cells were either J11d⁺ or J11d⁻ (panel c). In this experiment, none of the CD3⁺ cells carried IL-2R.

Methods. Cells were stained with J11d-FITC or PC61 (anti-IL-2R)-FITC plus arsenyl-conjugated F23.1 or arsenyl-conjugated T500 (anti-CD3), followed by a phycoerythrin (PE) coupled rabbit anti-arsenyl antiserum (arsenyl conjugates and anti-arsenyl antiserum were generously provided by J. Kimura and J. P. Allison).

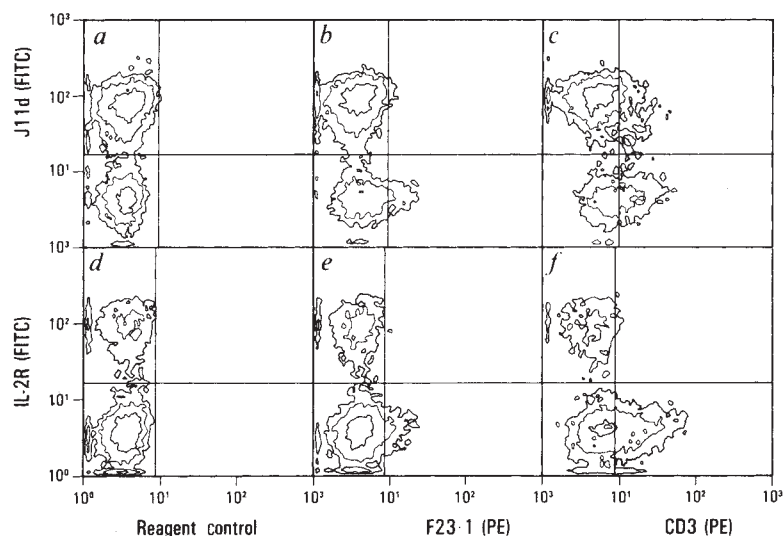
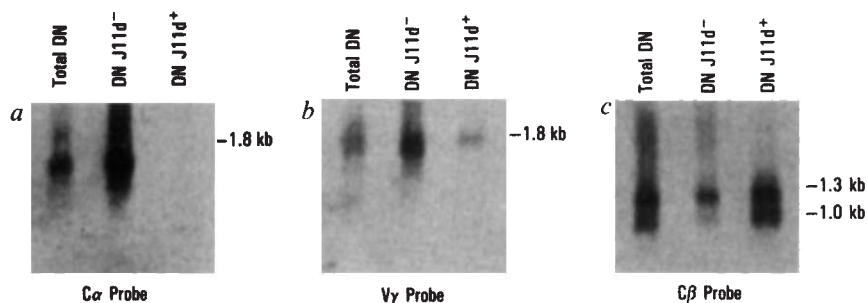


Fig. 3 Expression of α -, β -, and γ -chain mRNA by the J11d⁻ and J11d⁺ subsets of CD4⁺CD8⁺ thymocytes prepared from adult (3-6 month old) BALB/c mice. C_β and V_γ mRNA were expressed in both subsets, but C_α mRNA was absolutely restricted to the J11d⁻ cells. The C_β mRNA in J11d⁻ cells was mainly 1.3 kb; all other cell populations had approximately equal amounts of 1.3 kb and 1.0 kb mRNA.

Methods. Purified subpopulations of CD4⁺CD8⁺ thymocytes were prepared by antibody and complement lysis, or by sorting on a FACStar (Becton-Dickinson). After sorting, cells were reanalysed on the same flow cytometer to determine purity. Cell preparations >97% homogeneous were used to make RNA for Northern analysis. Total cellular RNA was isolated using the guanidinium/CsCl procedure⁴¹ with typical yields of 1 μ g of total RNA from 10⁶ CD4⁺CD8⁺ thymocytes. Total RNA (5 μ g) was electrophoresed on 1% agarose-formaldehyde gels²² and transferred to nitrocellulose filters. Hybridizations to ³²P-labelled nick-translated²³ DNA probes for C_β (p86T5-ref. 24), C_α (p α 8(0.8) ref. 25), C_γ 2 (pSP6- γ C6 ref. 9) and V_γ 2 (pGp 17 γ V ref. 9) were performed overnight at 42 °C in 10% Dextran sulphate, 40% formamide, 4 $\times$ SSC, 10 mM Tris-HCl, 1 $\times$ Denhardt's and 50 μ g ml⁻¹ salmon testis DNA. Filters were washed at 68 °C with 0.1 $\times$ SSC containing 0.1% SDS, and autoradiograms were made at -70 °C using a DuPont Lightning Plus intensifying screen. Hybridization probes were removed by boiling the filters for 20 min in 10 mM Tris-HCl pH 7.5, 0.1% SDS and 1 mM EDTA.



that α -chain mRNA was found exclusively in the J11d⁻ subset of CD4⁺CD8⁺ cells. The expression of γ -chain mRNA was studied using a C_γ probe (data not shown) and a V_γ 2 probe which detects the γ -chain gene preferentially used in CD3 γ cells⁹. Both probes detected full-length γ message in both J11d⁺ and J11d⁻ cells. The C_β probe detected mRNA in both J11d⁺ and J11d⁻ cells, but in the latter cell type almost all of the message was 1.3 kb in size, whereas in unfractionated CD4⁺CD8⁺ cells and J11d⁻ cells (and in unselected thymocytes and a cytotoxic T-cell clone, data not shown) the same C_β probe hybridized to equivalent amounts of 1.3-kb and 1.0 kb mRNA (Fig. 3c).

The expression of CD3 $\alpha\beta$ antigen receptors on a subset of CD4⁺CD8⁺ 'early' thymocytes might suggest that these cells are differentiation intermediates, potentially subject to repertoire selection. On this basis one might expect them to be most abundant early in life, when thymocytopoiesis is maximal. But when we stained BALB/c thymocytes from mice of different ages, F23.1⁺ CD4⁺CD8⁺ cells were absent (<1% of CD4⁺CD8⁺) in neonates, uncommon (3-10% of CD4⁺CD8⁺) in 4-6-week young adult mice, and more common in much older mice (up to 20% of CD4⁺CD8⁺ cells in 12-14-week-old mice). Correspondingly, J11d⁻ CD4⁺CD8⁺ cells were rare in neonates, and made up >50% of CD4⁺CD8⁺ cells in older mice (data not shown). We found the appearance of J11d⁻ cells late in ontogeny difficult to reconcile with a role as early differentiation intermediates, although their expression of CD3 $\alpha\beta$ receptors clearly places them in the T-cell lineage. To clarify their status, the differentiation potential of the J11d⁺ and J11d⁻ subsets of CD4⁺CD8⁺ thymocytes was studied more directly.

Precursor cells from a variety of sources will differentiate into

thymocytes with all of the CD4/CD8 phenotypes found in the normal thymus, if they are allowed to recolonize fetal thymus lobes, from which all haematopoietic-derived cells have been depleted by culture in 2-deoxyguanosine¹⁰. We used this system to examine the differentiation potential of the J11d⁺ and J11d⁻ subsets of CD4⁺CD8⁺ thymocytes, prepared from BALB/c thymocytes by antibody and complement depletion and sorting on a FACStar cell sorter (Fig. 4a, b). Fetal thymus lobes recolonized with J11d⁺ cells contained cells with CD4⁺CD8⁺ and CD4⁺CD8⁺ 'medullary type' surface phenotypes, and CD4⁺CD8⁺ 'cortical type' cells (Fig. 4c). These cell populations closely resemble those which develop in unmanipulated fetal thymus organ cultures². In contrast, lobes recolonized with J11d⁻ cells contained mostly CD4⁺CD8⁺ cells, plus a few cells expressing low levels of CD8 (Fig. 4d). The 'cortical-type' CD4⁺CD8⁺ phenotype, which predominates among normal thymocytes, was strikingly absent from the organ cultures. The surface phenotypes of the progeny of both J11d⁺ cells and J11d⁻ cells did not change in any systematic way between the seventh and the fifteenth day *in vitro*. On this basis, it is difficult to sustain the idea that J11d⁻, CD4⁺CD8⁺ cells are early progenitors which first acquire J11d, then diversify to give rise to all other thymocyte phenotypes.

Because differentiation in organ culture occurs in the absence of other haematopoietic-derived cell types, we were concerned that the failure of J11d⁻ cells to display progenitor activity might reflect a requirement for an unknown cell interaction which the deoxyguanosine-depleted lobes were unable to provide. We therefore performed experiments of the same kind *in vivo*, using intrathymic adoptive transfer to abrogate any requirement for cell homing¹¹, and the Thy-1 congenic mouse strains C57BL/6

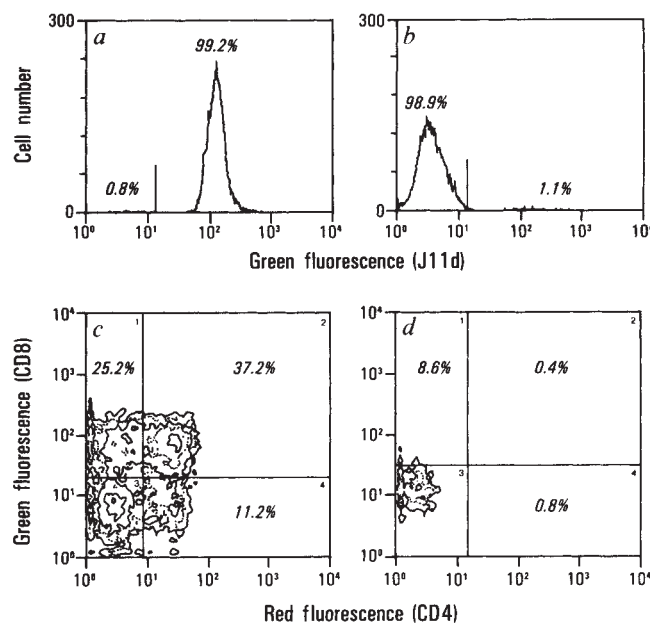


Fig. 4 Differentiation potential of $CD4^{-}8^{-}$ subsets over 9 days in organ culture.

Methods. $CD4^{-}8^{-}$ cells were made by two cycles of antibody and complement treatment, then stained with J11d and FITC anti-rat immunoglobulin. Cells were first analysed and then sorted, on a FACStar (Becton-Dickinson) employing a 2-W argon laser routinely operated at 250 mW, using a sorting rate of 1,000–2,000 cells per second, and excluding around 10% of the cells with intermediate levels of fluorescence. Cells were maintained in cold ($0-4^{\circ}C$) sterile media without azide throughout the staining and sorting operations. Fetal mice at day 14–15 of gestation were dissected under sterile conditions. Their thymus lobes were cultured in deoxyguanosine by a method based on that of Jenkinson *et al.*¹⁰. Briefly, groups of 4–6 individual lobes were cultured on nitrocellulose filters (Nuclepore) supported by gelatin sponges (Gelfoam, Upjohn) in RPMI 1640 medium (M.A. Bioproducts) containing 10% FCS plus glutamine and antibiotics. This culture system supports the thymus lobes at a medium-gas interface. To this medium was added 2-deoxyguanosine (Sigma) to a final concentration of 1.35 mM. After 5 days of culture in a $37^{\circ}C$, 5% CO_2 incubator, thymus lobes were transferred to a large volume of RPMI 1640 plus 5% FCS for 2 h, to allow deoxyguanosine to diffuse away. The lobes were then transferred to Terasaki wells containing 2.0×10^4 sorted $J11d^{+}$ or $J11d^{-}$ $CD4^{-}8^{-}$ cells, in RPMI culture medium without deoxyguanosine. The Terasaki plates were inverted so that recolonization occurred in a hanging drop. After 2 days, fetal thymus lobes were returned to nitrocellulose filters on gelatin foam rafts for a further 5–13 days. Groups of thymus lobes were disrupted in a miniature tissue grinder to obtain cell suspensions. Pooled cells from 5–10 thymus lobes (usually 10^4 – 10^5 cells) were stained and analysed on the FACS IV.

(Thy-1.2) and B6.PL.thy-1^a (Thy-1.1) as host and donor respectively. The subpopulations of C57BL/6 $CD4^{-}8^{-}$ thymocytes are the same as BALB/c with respect to surface phenotypes, but on average $J11d^{-}$ cells are fewer and IL-2R⁺ cells correspondingly more frequent in C57BL/6 (data not shown).

Cells prepared by complement-mediated lysis, staining and sorting were injected into the thymuses of lethally irradiated, Thy-1 congenic hosts. These host mice received sorted $CD4^{-}8^{-}$ cells in 10 μ l of saline into each thymus lobe, and an intravenous cell transfer of Thy-1-depleted bone marrow syngeneic with the host. After intrathymic injection, donor cells rapidly recolonize the thymus and are detectable by staining for the donor Thy-1 allele at 5–15 days post-transfer. After this time, they are replaced in the thymus by host-type cells derived from the syngeneic bone marrow transfer. After 10 days, they begin to seed the peripheral lymphoid organs with donor-derived T cells²⁷.

Table 1 Recolonization of the thymus and spleen after intrathymic adoptive transfer of subsets of $CD4^{-}8^{-}$ thymocytes

Time after adoptive transfer	Organ	Stain	Donor cells		
			J11d ⁺	J11d ⁻	None
12 days	Thymus	Thy-1	85.1	80.5	94.6
		Thy-1.1	25.9	0.2	0.1
19 days	Thymus	Thy-1	95.9	92.2	95.5
		Thy-1.1	4.3	0.0	1.1
	Spleen	Thy-1	6.5	5.7	6.3
		Thy-1.1	3.0	0.0	0.0

Percentage of total T cells and donor-derived T cells in mice recolonized with sorted $CD4^{-}8^{-}$ thymocytes. Host B6 mice were irradiated with 950 rad, 2–4 h before cell transfer. Syngeneic, T-depleted bone marrow was prepared by treatment of femoral and tibial bone marrow cells with anti-Thy-1 (T24) and complement. Host mice received 5×10^6 syngeneic bone marrow cells intravenously 1–2 h before intrathymic transfer. Irradiated, bone marrow reconstituted mice were anaesthetized with tribromoethanol. The thymus was exposed, and 10 μ l of HBSS containing precursor cells was injected into each thymus lobe. The incision was closed with surgical clips, and animals were maintained on antibiotic water but otherwise under normal vivarium conditions. At 12 and 19 days after cell transfer single-cell suspensions were prepared from thymus and spleen. Cell yields per host were approximately 100×10^6 thymocytes from all experimental groups at both time points, and around 120×10^6 spleen cells from all groups at day 19. Cells were stained with T24 (anti-Thy-1) and 19E12 (anti-Thy-1.1)²⁶, and analysed on the FACS IV to identify total and donor-derived T cells.

In the experiment summarized in Table 1, 1.0×10^6 $J11d^{+}$ cells (98.6% $J11d^{+}$ on re-analysis) recolonized the thymus, forming 25.9% of the cells at 12 days post-transfer, and declining to 4.3% by 19 days. At 19 days, around 6% of the spleen was Thy-1⁺ and half of these T cells were donor-derived. In sharp contrast, the hosts which received an equal number of $J11d^{-}$ cells (99.1% $J11d^{-}$ on re-analysis) did not show repopulation of either the thymus or the spleen. We are confident that the sensitivity of the assay did not limit detection of precursor cells, because in other experiments we could detect the progeny of 4.0×10^4 $CD4^{-}8^{-}$ cells, or 2.0×10^4 $J11d^{+}$ $CD4^{-}8^{-}$ cells which made up 8.8% and 5.0%, respectively, of the regenerating thymus at 10 days).

In fetal thymus organ culture, and in the irradiated host *in vivo*, the ability of $CD4^{-}8^{-}$ thymocytes to act as progenitor T cells is found exclusively among $J11d^{+}$ cells. What then is the status of the $J11d^{-}$ $CD4^{-}8^{-}$ cells? Several pieces of evidence suggest that they represent a pathway which diverges from the main line of T cell differentiation. First, they express an aberrant receptor repertoire in which F23.1⁺ β -chains, belonging to a single three-member V_{β} gene family¹², are expressed at unusually high frequency. Second, we found a great excess of 1.3-kb over 1.0-kb β -chain mRNA in these cells, which might result from V-D-J rearrangements on both chromosomes (although other explanations are possible). Third, they lack K⁺ channels, in contrast to other thymocytes¹³. Fourth, they are rare until long after the peak of thymocytopoiesis. But we do not know whether they are a functional lineage, or dead-end cells.

Our results also touch on the question whether $CD3\alpha\beta$ and $CD3\gamma\delta$ cells have a common intrathymic precursor^{14,15}. As we find surface CD3 and γ message in the same subset of $CD4^{-}8^{-}$ cells as all precursor T-cell activity, a common precursor cell type would presumably have that subset's phenotype (Thy-1⁺ $J11d^{+}$ $CD4^{-}8^{-}$). Our data do not, however, rule out the possibility that cells which actually express γ protein subsequently differentiate into T cells^{9,16}.

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A novel HLA class II molecule (DR α –DQ β) created by mismatched isotype pairing

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Human major histocompatibility complex (MHC) class II molecules are heterodimeric glycoproteins composed of non-covalently associated α and β chains¹. Only isotype-matched α – β associations have been described in man; these can occur either by *cis*- or *trans*-complementation (HLA–DR, DQ, DP)². Here evidence is provided for the existence of a new type of hybrid molecule (DR α –DQ β) arising by mixed-isotype pairing in human B-cell lines. Class II isotype-mismatched heterodimers have been recently reported in the mouse after transfection of class II genes^{3–5}, and our data demonstrate that such interisotypic pairing can occur in untransfected cells. This crosspairing greatly enhances the repertoire of the class II antigens that regulate immune responses and leads us to reconsider the HLA-disease association.

The homozygous EBV-transformed B-cell line PGF was radio-labelled with ³⁵S-methionine⁶. HLA-DR and HLA-DQ molecules were immunoprecipitated and analysed by two-dimensional gel electrophoresis. D1–12 (a monoclonal antibody, ref. 7 anti-DR) precipitates two DR2 β -chains in addition to the DR α -chain⁸ (Fig. 1a). The G2a5 immunoprecipitate (a mutant of Genox 3–53 specific for DQw1 molecules) revealed the presence of a DQw1 β , DQw1 α complex⁹ (Fig. 1b). No DR β -chain is found in this profile. The comigration of D1.12 and G2a5 immunoprecipitates showed that no β -region spots are shared between DR and DQ (Fig. 1c). But an additional spot with acidic migration and slightly higher molecular weight than the DQw1 α -chain is also detected in the G2a5 immunoprecipitate (Fig. 1b). Two series of spots are observed in the G2a5

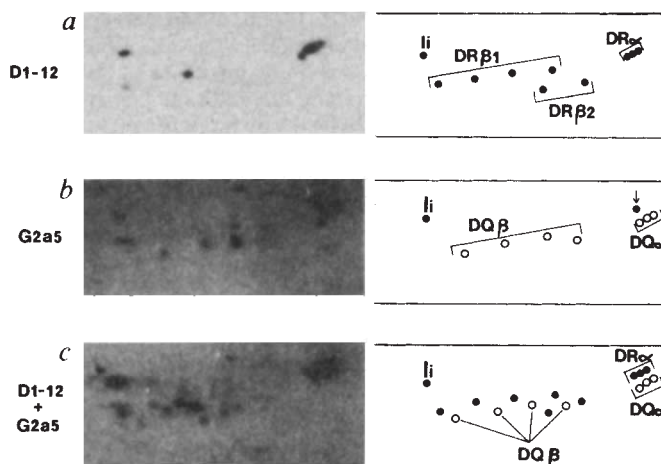


Fig. 1 Comparative two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) profiles of DR β -chain and DQ β -chain from PGF (DR2–DRw2–DQw1) DR2 β -chain and DR2 β -chain immunoprecipitated by D1–12 (anti-DR antibody). *b*, Immunoprecipitation of DQ molecules by the monoclonal antibody G2a5 (anti-DQw1 antibody). The vertical arrow indicates an additional spot which is slightly higher than the DQw1 α -chain. Spots not referred to in the diagram are background. Comigration and depletion were performed to demonstrate that these spots are not DR and that the DR α -chain (vertical arrow) can only be associated with the DQw1 β -chain (Figs 1c and 3). *c*, Comigration of D1.12 and G2a5 immunoprecipitates.

Methods. Internal labelling and immunoprecipitations were performed as previously described²⁰. Briefly, 10^7 cells were cultured with 0.1 mCi of ³⁵S-methionine for 5 h. The membranes were extracted with 0.5% Nonidet P-40. The two-step precipitates were run in 2D-PAGE with NEPHGE for the first dimension and SDS-PAGE (10% acrylamide gel) in the second dimension.

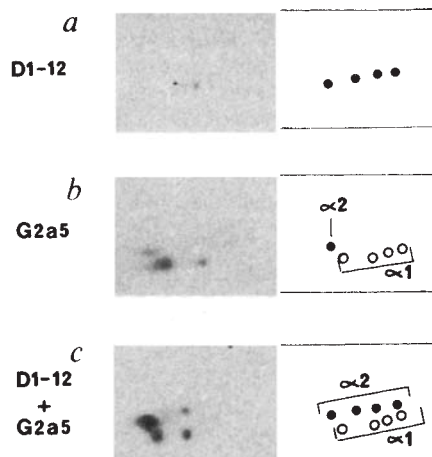


Fig. 2 Comparative isoelectric focusing 2D-PAGE profiles of DR α -chain and DQw1 α -chain from PGF. *a*, DR α -chain immunoprecipitated with D1–12; *b*, G2a5 immunoprecipitate. An additional spot (vertical arrow) is revealed in addition to the DQw1 α -chain. Sialylated spots are very weak because of the low amount of this chain and are not referred to in the diagram. *c*, Co-migration of D1–12 and G2a5 immunoprecipitates. The more basic spot of the D1.12 immunoprecipitate appeared indistinguishable from the α 2-chain spot of the G2a5 immunoprecipitate. Moreover this α 2-chain spot has increased in intensity indicating that the two spots comigrate to exactly the same position. Radiolabelling and immunoprecipitations were as for in Fig. 1. The 2D-PAGE was performed with isoelectric focusing in the first dimension.

immunoprecipitate (Fig. 2). The first, α 1, corresponds to the DQ α -chain with successive additions of sialic acid residues. The second, α 2, consists of a more basic spot (sialic acid addition is apparent after longer exposure). When compared with the

DR α -chain precipitated by D1-12, the α_2 chains appear identical in both *pI* and molecular weight (Fig. 2a). When D1-12 and G2a5 immunoprecipitates were mixed (Fig. 2c) it was confirmed that the α_2 spot comigrates with the first spot of the DR α -chain whereas the DQw1 α -chain migrates slightly below. Therefore we postulated that a DR α -chain was present, although in low amounts, in the G2a5 immunoprecipitate.

In Western blot experiments the monoclonal antibody HC2-1 (specific for DR α)¹⁰ detected DR α -chain in a G2a5 immunoprecipitate (data not shown). We confirmed that the DR α -chain was associated with the DQ β -chain but not the DR β -chain as follows. After complete removal of the DR α - β complex by successive D1.12 immunoprecipitations, G2a5 still immunoprecipitated a DR α -chain recognized by HC2-1 (Fig. 3g, h, i). Western blots show that G2a5 recognizes an epitope on the DQw1 β -chain (Fig. 3a, f). Therefore the presence of the DR α -chain but not the DR β -chain in G2a5 precipitate indicates that the DQw1 β -chain can pair with the DR α -chain, generating a mixed-isotype heterodimer.

The mixed-isotype heterodimer can also be detected by an anti-DR α -chain monoclonal antibody. The radiolabelled homozygous B-cell line GM 3163 (ref 12) was immunoprecipitated with D1.12 (anti-DR antibody), CA-206 (anti DR β + DQB antibody¹³ and DA-6-147 (anti-DR α antibody). Figure 4a shows that two DR β -chains are expressed. The DQw2 β -chain is identified by three basic spots and the DQw2 α -chain has a slightly lower molecular weight and a more acidic migration than the DR α -chain (Fig. 4b). Both DR and DQ β -chains were revealed with DA-6-147, although the DQ β -chain spots were less intense, but no DQ α -chains were present (Fig. 4c). No DQ α -chain antibody was detected in DA-6-147 immunoprecipitate using a series of monoclonal anti-DQ α -chain antibodies in Western blot experiments or in isoelectric focusing patterns (data not shown). These results indicate that there is no cross-reaction between DR α and DQ α and strongly suggest that a subset of the DQ β -chain is associated with the DR α -chain. Furthermore, complete removal of DR α -chain-associated β -chains by successive precipitations with DA-6-147 leaves the DQ α - β complex, detectable with CA-206 (Fig. 4d). These data indicate that there are no free DR β -chains which would otherwise be bound by CA-206; all the DR β -chains, must be paired to the DR α -chains as they were completely removed by DA-6-147 depletion. Therefore there is no DQ α -DR β molecule,

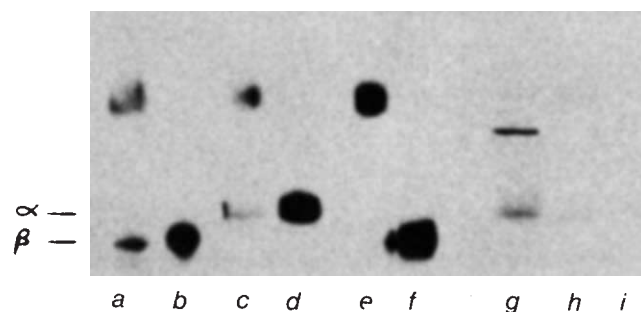


Fig. 3 Anti-chain reactivity of G2a5. Extract of the cell line SC-CA (DR1, DQ1) was separated by SDS-PAGE and transferred to nitrocellulose a, c, e, extracts not boiled; b, d, f, extracts boiled with 2.3% SDS. Lanes a and b were probed with the antibody SG-171 (binding to the DQw1 β -chain, ref. 11), lanes c and d with the antibody DA-6-147 (which reacts with the DR α -chain) and lanes e and f with antibody G2a5. In a and c the SDS has dissociated the chains without boiling. The cell line SC-CA was routinely used to test a panel of monoclonal antibodies; we confirmed the specificity of these antibodies using several other cell lines. g-i, precipitation of DR α -chain by HC2-1 from sequential immunoprecipitates using D1.12 and from an immunoprecipitate using G2a5. g, first D1.12 immunoprecipitate; h, G2a5 immunoprecipitate from extract precleared six times with D1.12, sixth D1.12 immunoprecipitate.

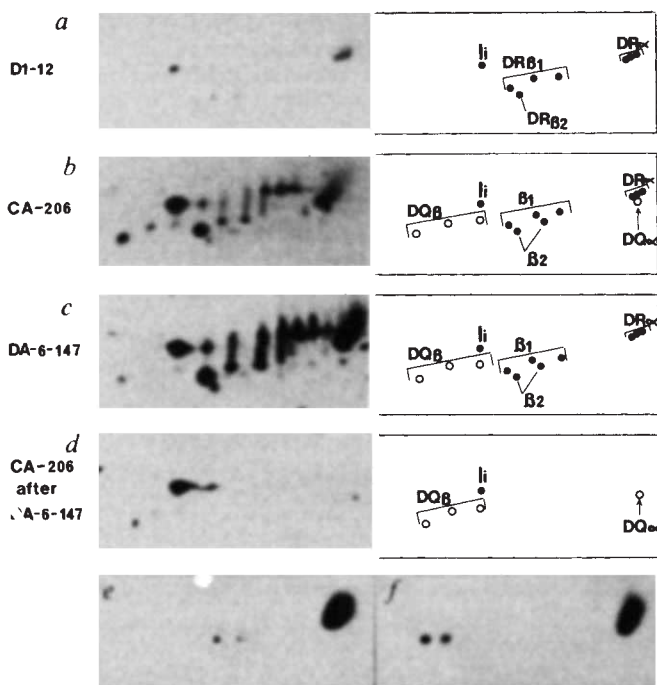


Fig. 4 Comparative 2D-PAGE profiles of DR β -chain and DQ β -chain from GM 3163 (DR7/DQw2). a, D1.12 immunoprecipitate, showing the classical pattern of DR7 β -chains¹². b, CA-206 (anti-DR β -chain and DQ β -chain) recognizes the two DR β -chains and three basic spots corresponding to the classical DQw2 β -chain¹². The DQw2 α -chain shows a slightly lower molecular weight and a more acidic migration than the DR α -chain (vertical arrow). c, The same β -chain pattern was obtained with the monoclonal antibody DA-6-147 (anti-DR α -chain antibody) although the DQw2 β -chain is faint compared to the DQw2 β -chain spots from CA-206 immunoprecipitate. In contrast no DQ α -chain was detectable in DA-6-147 immunoprecipitate. d, After depletion of DA-6-147 reactivity by six successive precipitations (not shown) CA-206 can still precipitate the DQw2 α , β , β complex. e, Cells were surface-labelled with ¹²⁵I by the lactoperoxidase, catalysed iodination method²⁰. D1-12 precipitates the two DR β -chains and the DR α -chains from the cell surface. The precursor of each chain is not present, indicating that there is no cytoplasmic contamination. f, After depletion of the DR complex by six successive D1-12 precipitations DA-6-147 can still precipitate the DR α -DQ β molecule from the cell surface.

indicating that the mixed isotype pairing is not due to a technical artefact as it is not observed in all combinations. Pious *et al.* also suggested that the DR β -chain cannot pair with the DQ α -chain or the DP α -chain¹⁵. In Fig. 4e the DR α -DQ β molecule was precipitated by DA-6-147 from the cell surface after depletion of the DR α - β complex.

Earlier studies have demonstrated the interspecies combinatorial association of α - β chains in hamster B cells transfected with murine I-A^k genes¹⁶. Norcross *et al.* have reported the pairing of DR α and I-A β^d chains after transfection of the murine A β^d gene into a human EBV-transformed cell line¹⁷. Transfection of murine I-E and I-A genes into L cells has demonstrated mixed-isotype pairing, giving an I-A β -IE α complex³⁻⁵. Transfection cannot conclusively demonstrate that these mixed-isotype pairing would occur in unmanipulated cells, however. Cross-association of I-A and I-E in the mouse has been suggested¹⁸. Here we show that such cross-isotype pairing can occur in untransfected cells, providing physiological relevance for the transfection results.

Cross-isotype pairing appears to be highly influenced by the allelic polymorphism of the I-A β chain as the I-A β -IE α complex could not be detected in all combinations, indicating that the polymorphic NH₂ terminal region I-A β is important³.

Haplotype-mismatched associations also seem to be controlled by the polymorphic NH₂-terminal domain and need high levels of messenger RNA¹⁹. The cross-isotype pairing in human cells could also be influenced by at least two factors. First, the DQ β -chain polymorphism could influence pairing with the DR α -chain. Second, in permissive haplotypes mixed-isotype heterodimers could be the result of unfavorable pairing occurring after all the favoured, isotype-matched associations. The amount of such unorthodox associations may vary during the upregulation of class II expression by lymphokines.

It has been shown in the mouse that interisotypic heterodimers can present antigen but it is not yet known whether they are functional in humans⁵. Mixed-isotype α - β pairing could explain the fact that certain diseases are more highly correlated to haplotype than isotype.

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Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA

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E-cadherin is a cell surface glycoprotein responsible for Ca²⁺-dependent intercellular adhesion between epithelial cells¹; it is also called uvomorulin², L-CAM (ref. 3), cell-CAM 120/80 (ref. 4) or Arc-1 (ref. 5). Because blocking the action of E-cadherin by monoclonal antibodies causes dispersion of compact cell colonies¹, this molecule is thought to be an important factor for maintenance of multicellular systems. To demonstrate directly that E-cadherin is involved in cell-cell adhesion, we cloned full-length cDNA encoding E-cadherin from F9 cells and introduced it into L fibroblasts deficient in E-cadherin. These L cells acquire strong Ca²⁺-dependent aggregating activity by expressing the E-cadherin derived from the introduced cDNA, and were morphologically

transformed so as to form colonies in which cells were tightly connected to each other.

Complementary DNA libraries constructed from the messenger RNA of mouse teratocarcinoma F9 cells expressing E-cadherin were screened for a positive reaction to a polyclonal antibody against E-cadherin. Northern blot analysis using one of the positive cDNA clones detected one band of ~4.5 kilobases (kb) from F9 cells, but did not detect it in L fibroblasts (Fig. 2a). This size of mRNA is long enough to encode the putative precursor, of molecular mass 135,000 (*M_r* 135K), of the E-cadherin peptide^{6,7}. We then rescreened the libraries to cover the entire region encoding E-cadherin mRNA. Clones thus obtained were sequenced and assembled into a full-length cDNA.

Figure 1 shows the complete nucleotide sequence of the full-length cDNA with its predicted amino-acid sequence. Within this sequence, we detected the same sequence of 25 amino acids as at the amino-terminus of the *M_r* 84K fragment of the mature liver E-cadherin as determined using a gas-phase amino-acid sequencer⁸. The only exception was that Ser-Cys is substituted for Val-Val in the amino-acid sequence deduced from the nucleotide sequence. We found a consensus sequence for the translation initiation site⁹; followed by a hydrophobic peptide sequence that is probably the signal peptide¹⁰; a highly hydrophobic region which probably corresponds to a transmembrane domain of this protein; and a potential polyadenylation signal¹¹. These results suggest that the cDNA clone encodes E-cadherin, and covers the whole coding region of the mRNA. Note that the predicted primary structure of E-cadherin peptide is similar to that of L-CAM, a chicken homologue of E-cadherin described recently¹²; we found about 65% similarity between peptide sequences of these proteins derived from different animal species. Computer searches of sequence data bases detected no significant similarity between E-cadherin and other published protein sequences. A more precise analysis of the structure of E-cadherin and a comparison with that of other cadherins will be described elsewhere.

The full-length cDNA inserted into an eukaryotic expression vector was transfected into L cells, which do not express E-cadherin (Figs 2 and 3c), together with the gene for resistance to the antibiotic G418. Stable transformants were selected by culturing in the presence of G418. From >40 G418-resistant clones, we selected several which reacted with ECCD-2, a monoclonal antibody against E-cadherin. As a control, we saved a few clones resistant to G418 that did not react with the ECCD-2 antibody.

Northern blot analysis showed that L-cell transformants reacting with ECCD-2 have a major RNA species of 5.1 kb which hybridizes with the E-cadherin cDNA probe. Its size corresponds to that of the exogenously introduced cDNA but not to that of endogenous E-cadherin mRNA detected in F9 cells (Fig. 2a). Immunoblot analysis showed that these L-cell transformants express a major 124K protein and other minor proteins which react with ECCD-2; the size of the major component is the same as that of E-cadherin detected in F9 cells (Fig. 2b). G418-resistant clones not reacting with ECCD-2 (such as EL5) were negative in these tests.

Table 1 shows that L-cell transformants reacting with ECCD-2 acquired strong Ca²⁺-dependent aggregating activity. The extent of the aggregating capacity was closely correlated with E-cadherin expression (compare Fig. 2 and Table 1). The aggregation of these transformants was inhibited by the monoclonal antibody ECCD-1, known to block specifically the action of E-cadherin (Table 1). E-cadherin-negative transformants (such as EL5), as well as normal L cells, did not aggregate significantly under these conditions. These results clearly show that E-cadherin molecules expressed in L-cell transformants confer aggregating activity on the cells.

Interestingly, most of the L-cell transformants expressing E-cadherin were morphologically transformed. Generally, L

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Fig. 1 E-cadherin cDNA sequence. *a*, Restriction map and cDNA fragments. Restriction sites: B, *Bam*HI; E, *Eco*RI; P, *Pvu*II; S, *Sac*I. Open box, E-cadherin coding region. *b*, Nucleotide and predicted amino-acid sequence of the E-cadherin cDNA clone EM1. An open reading frame of 2,652 base pairs (bp) and the predicted sequence of 884 amino acids, as well as 68 bp of 5' untranslated sequence and 1,642 bp of 3' untranslated sequence containing a stretch of seven A nucleotides on the 3' terminus are shown. The ATG codon at position 69, which conforms to the consensus sequence for the initiation codons⁹, was the possible candidate for the initiation codon. This initiation codon is followed by a peptide (underlined) consisting of predominantly hydrophobic amino acids, which may work as a signal peptide. The boxed amino acid sequences for residues 157–181 correspond to those determined by amino-acid sequencing of the amino terminus of mouse liver E-cadherin 84K fragments⁸. A segment with 34 hydrophobic amino acid residues (presumably a transmembrane domain) has a heavy underline. The broken underline at positions 4,333–4,338 indicates a polyadenylation signal.

Methods. All clones were isolated from λgt11 expression libraries of mouse F9 cDNA constructed using a random or oligo(dT) primer. Clone λ04 was obtained as a clone reacting with the polyclonal antibody raised against 84K fragments of the mouse liver E-cadherin⁷; λ13 and λ21 were cloned by hybridization with the λ04 probe. A full length cDNA (EM1) was constructed by connecting λ13 and λ21 at the *Pvu*II site. The nucleotide sequence was determined by the dideoxy method¹⁵. Analysis of DNA sequence data was carried out using the DNASIS program (Hitachi).

Fig. 2 Expression of E-cadherin cDNA transfected into L cells. *a*, Northern blot hybridization of total RNA isolated from F9 cells, normal L cells, and G418-resistant transformants (EL4, EL8, EL2, EL25 and EL5). In F9, a 4.5-kb RNA species was hybridized, and in all E-cadherin-positive transformants (see *b*), the largest band detected was one of 5.1 kb; 5.1 kb corresponds to the predicted size of transcripts of the exogenously introduced cDNA. L cells and E-cadherin-negative transformant EL5 showed no corresponding band. *b*, Immunoblot detection of E-cadherin by ECCD-2 antibody from the same cells as in *a*.

Methods. The plasmids pSTneo (for G418 resistance) and pSTEM1 (for E-cadherin cDNA expression) were co-transfected to L cells at the molar ratio 1:10 by the calcium phosphate method¹⁶, and stable transformants were selected by G418 resistance¹⁷. Plasmid pSTneo is a derivative of pSV2neo (ref. 18) in which a simian virus 40/herpes simplex virus thymidine kinase gene (SV40-HSVtk) composite promoter¹⁹ is used; pSTEM1 was constructed from pSTneo by replacing the coding region for G418 resistance (between the *Bgl*III and *Sma*I sites) with the 3.9-kb 5' part of the EM1 sequence. The transformants were screened for positive antibody reaction to E-cadherin by immunofluorescent cytochemistry. Northern blot analysis was carried out using formaldehyde gels as described²⁰. Blots on a nitrocellulose membrane (10 µg RNA per lane) were probed with ³²P-labelled clone λ04. Immunoblot analysis using ECCD-2 antibody was carried out as described⁷.

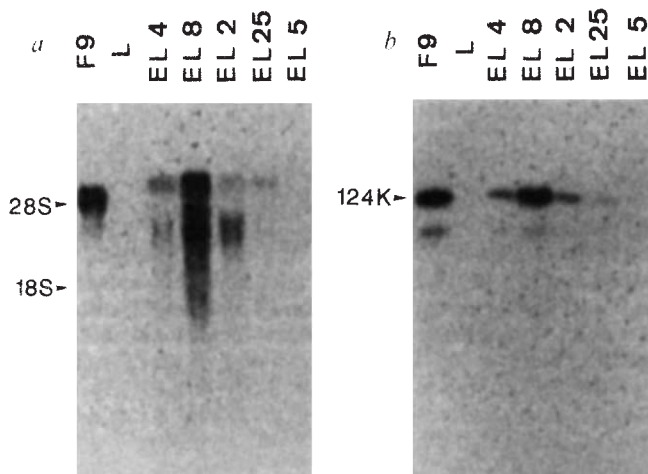


Table 1 Ca^{2+} -dependent aggregation of untransformed and transformed L and F9 cells

Cells	Degree of aggregation (N_i/N_0)		
	without Ca^{2+}	with Ca^{2+}	with Ca^{2+} and ECCD-I
L	0.96	0.96	0.86 (—)
EL4	0.97	0.46	0.95 (91.1)
EL8	0.99	0.04	0.84 (83.3)
EL2	0.99	0.61	1.00 (100)
EL5	1.00	1.02	0.91 (—)
F9	0.98	0.21	0.90 (87.0)

Cells were dissociated by treatment with 0.01% trypsin in the presence of 1 mM Ca^{2+} leaving E-cadherin intact, and allowed to aggregate for 30 min in the absence or presence of 1 mM Ca^{2+} under the conditions described previously¹⁴. The effect of ECCD-1 was examined by adding 200 $\mu\text{g ml}^{-1}$ of this antibody to the aggregation medium as described¹; as a control, the effect of the ECCD-2 antibody, which is known to be unable to block E-cadherin action, was tested: no inhibition was found. Degree of aggregation was expressed as N_i/N_0 , where N_0 is the initial particle number of the cell suspension and N_i is the particle number after 30 min incubation (see ref. 14 for method). Values in parentheses are percentage inhibition of aggregation by ECCD-1, calculated as described¹⁴; — indicates that no significant aggregation occurred.

cells do not form tight intercellular connections in monolayer cultures; they seem to be independent and criss-cross on the culture substratum as if not affected by each other (Fig. 3a). However, L-cell transformants expressing E-cadherin were always connected to each other at their peripheries (Fig. 3b), although the morphology of their colonies varied between lines. When these L-cell transformants were immunohistochemically stained for E-cadherin, the antigens were found to be concentrated in the boundary between cells (Fig. 3d), as in cells endogenously expressing E-cadherin. Thus, the adhesive behaviour of L cells was obviously transformed by expressing E-cadherin.

These results provide the first direct evidence that E-cadherin is essential for intercellular adhesion. They also show that expression of a single class of peptide is sufficient to cause

Ca^{2+} -dependent cell-cell adhesion. The technology used successfully here might be applied to more complicated systems to investigate the function of E-cadherin and other cell adhesion molecules. For example, to determine the function of E-cadherin in animal morphogenesis, we may be able to perturb the developmentally regulated expression of this molecule¹³ by introducing E-cadherin cDNA with a foreign promoter into fertilized eggs. It may also be possible to examine the function of cadherins in the behaviour of malignant cells; cadherin cDNA can be introduced into malignant cells to see whether it affects their metastasizing capacity. Experiments of these types should initiate a new approach to studying mechanisms of cell behaviour in the animal body.

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Molecular cloning and sequencing of a human hepatitis delta (δ) virus RNA

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Human hepatitis delta (δ) virus (HDV) is a form of defective virus, which infects humans only in the presence of a co-infecting hepatitis B virus (HBV). HDV superinfection in a chronic HBV carrier often results in severe chronic hepatitis and cirrhosis, whereas acute HDV and HBV co-infection is frequently associated with fulminant hepatitis^{1–3}. HDV consists of a 36-nm particle, which contains an envelope with HBV surface antigen, and a nucleocapsid containing the hepatitis δ -antigen (HDAg) and an RNA genome of 1.75 kilobases (kb) (ref. 4). Recently, the genomic RNA from an HDV serially passaged in chimpanzees has been cloned and sequenced in a study which showed that the HDV RNA is a single-stranded circular molecule with properties similar to those of viroid or virusoid^{5,6}. However, it is not known whether serial passages in chimpanzees had altered the properties of human HDV. Here we report the cloning and sequencing of an HDV RNA isolated directly from a patient with acute δ -hepatitis. The

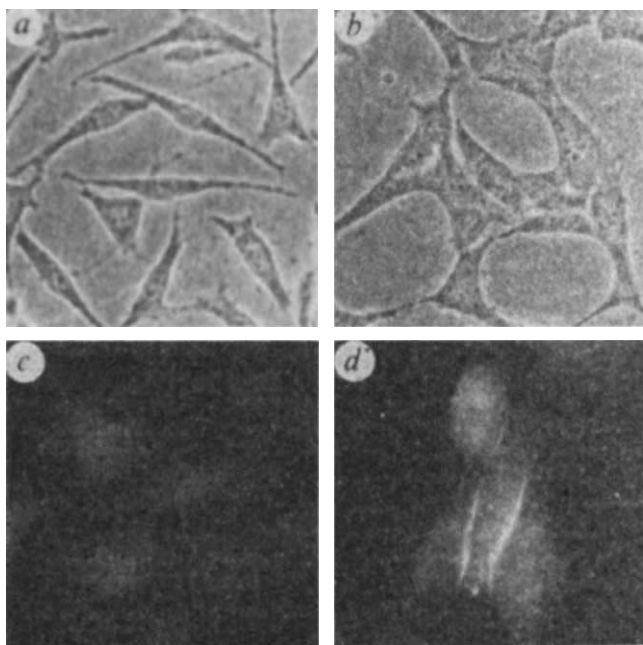


Fig. 3 Photomicrographs of untransformed L (a and c) and transformed EL8 (b and d) cells. a, b, Phase-contrast microscopy, $\times 125$; c, d, immunofluorescent staining for E-cadherin using ECCD-2 antibody, $\times 650$.

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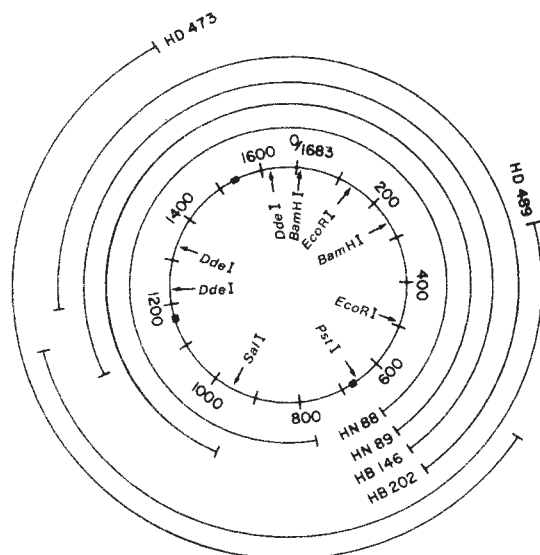


Fig. 1 Diagram of the HDV cDNA clones. In this map, the *Hind*III site of the chimpanzee HDV isolate⁵ is designated as nucleotide 0/1683, although this site is missing in human HDV isolate. Square boxes, positions of the primers used, which represent nucleotides 658–674, 1,171–1,187 and 1,515–1,528 respectively.

Methods. The HDV was purified from the patient's plasma by centrifugation in discontinuous sucrose gradients consisting of 60% (w/w) and 20% sucrose in NTE buffer (0.1 M NaCl, 0.01 M Tris-HCl, pH 7.2, 1 mM EDTA). After centrifugation at 39,000 r.p.m. for 5 h at 4°C in a Beckman SW41 rotor, the virus band at the interface between 60% and 20% sucrose was collected, diluted threefold with NTE buffer, and applied onto a second discontinuous sucrose gradient consisting of 60%, 50%, 40%, 30% and 20% sucrose. After centrifugation at 26,000 r.p.m. in an SW41 rotor for 13 h at 4°C, fractions containing 30–40% interface were collected. This fraction contained the HDag, as determined by enzyme linked immunosorbent assay (ELISA) test (data not shown). The virus fraction was diluted with NTE buffer and centrifuged at 40,000 r.p.m. in an SW41 rotor for 1.5 h at 4°C. The virus pellet was resuspended in NTE buffer containing 1% SDS. The RNA was extracted with phenol/chloroform/isoamyl alcohol (50:50:1), and precipitated with ethanol. The RNA was used directly for cDNA synthesis using three synthetic oligodeoxyribonucleotides as primers according to the procedures described in ref. 18. Second-strand cDNA synthesis was performed according to ref. 13. The DNA was dC-tailed and cloned into the *Pst*I site of dG-tailed pBR322. Transformation was performed by the calcium phosphate method into *Escherichia coli* strain MC1061 (ref. 19).

sequence showed considerable divergence (11%) from that of the chimpanzee-adapted HDV. Five open reading frames (ORFs) of more than 100 amino acids in both genomic and anti-genomic sense were found. The largest ORF in antigenomic sense, which can code for 214 amino acids, may correspond to the HDag.

HDV infection is prevalent in many parts of the world. The disease is endemic in Italy and in developing countries from the equator to the tropics. Several δ -hepatitis epidemics have been reported in the Amazon basin of South America. HDV infection is also prevalent among intravenous drug users and haemophiliacs: more than 50% of the HBV carriers among this population in the USA have HDV infection⁷.

To understand the pathogenesis and molecular biology of human HDV, we have cloned and sequenced an HDV RNA directly isolated from a patient with acute δ -hepatitis. This approach was in contrast to the previous studies which examined the chimpanzee-adapted HDV (refs 5, 8 and 9). HDV particles were isolated from a 40-year-old intravenous drug user in the initial stage of acute δ -hepatitis, when the patient showed a strong δ -antigenaemia. The virus was purified from plasma by discontinuous sucrose gradient sedimentation. HDV banded at the interface of 30% and 40% (w/w) sucrose, and viral RNA

was extracted by phenol-chloroform. The RNA was shown by electrophoresis on 1% agarose gel to consist of a single RNA band slightly smaller than the 18S ribosomal RNA marker (data not shown).

To clone HDV RNA molecularly, we first determined partial sequences of HDV RNA by RNA sequencing. For this purpose the RNA was digested with RNase T₁, end-labelled with [γ -³²P]ATP by polynucleotide kinase and the resulting oligonucleotides separated by two-dimensional polyacrylamide gel electrophoresis ('oligonucleotide fingerprinting')¹⁰. Individual T₁-oligonucleotides were eluted from the gel and sequenced by the partial enzymatic digestion method^{11,12} (data not shown). Two of the T₁-oligonucleotides had the sequences

5'-UAAUUCUUCUUUCCUUUUNNG-3'

and

5'-UUCCUCUAAACUUCUUUCUUNNG-3'

respectively. We therefore prepared two complementary synthetic oligodeoxyribonucleotides

(5'-AAAAGGGAAAGAAGAATA)

and

(5'-AGAAGTTAGAGGAA-3')

as the primers for complementary DNA synthesis. We also prepared a third primer

(5'-GGATGGAACGCGGACCCTG-3')

complementary to part of a reported chimpanzee HDV cDNA clone, pKD3 (ref. 9). These primers were mixed with the HDV RNA and used for reverse transcription. Second-strand DNA synthesis and subsequent cloning followed ref. 13.

The cDNA clones obtained were screened with these primers and also with the chimpanzee HDV-specific pKD3 clone⁹. More than 200 HDV-specific clones were obtained in which the largest clone was >1.5 kb. The restriction map of these clones was compatible with a circular structure for the HDV RNA (ref. 5, Fig. 1). We adopted the map designation of Wang *et al.*⁵, using the unique *Hind*III site as nucleotide 0, although the comparable *Hind*III site is missing from the human HDV cDNA. Nevertheless, the conservation of sequences around this site (see below) allowed us to align these two RNAs. Sequence analysis was carried out using three different cDNA clones and examined by both the dideoxy chain termination¹⁴ and the chemical modification methods¹⁵.

The complete sequence of the human HDV is shown in Fig. 2. Human HDV is 1,683 nucleotides in length and is present as a circular RNA molecule. The sequence was compared with that obtained from chimpanzee-adapted HDV (refs 5 and 6). The overall homology between the two sequences is 89%; however, there are domains of relative conservation and of more pronounced divergence. There are five ORFs able to encode more than 100 amino acids each, four of which are relatively conserved between the human and chimpanzee HDV isolates (Fig. 3). Note that two of the ORFs are in the genomic sense, whereas the other three are in the anti-genomic orientation. If all the ORFs are indeed translated into proteins, then the HDV RNA would represent a novel class of RNA, in which the same region of a single-stranded RNA is translated in both orientations. The most notable ORF is in the reading frame -2. This ORF has been implicated as the region encoding the δ -antigen⁵. Our human HDV sequence suggests a HDag of 214 amino acids with relative molecular mass (M_r) 24,000, which is highly conserved between the human and chimpanzee-adapted HDV isolates. This protein contains highly charged amino acids in the N-terminal two-thirds and uncharged amino acids at the C-terminus (data not shown). The abundance of highly basic amino acid residues suggests that the δ -protein interacts with the delta viral RNA. The ORF in the reading frame -3 is in the most diverged region of the HDV RNA, resulting in two potential proteins of almost

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Homology-dependent interactions in phage λ site-specific recombination

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General recombination shows a dependence on large regions of homology between the two participating segments of DNA. Many site-specific recombination systems also exhibit a dependence on homology, although in these systems the requirement is limited to a short region (less than 10 base pairs (bp))¹⁻⁹. We have used the *in vitro* phage λ integration reaction to study the role of homology in this model site-specific recombination system. We find that certain non-homologous pairings which are strongly blocked for complete recombination, nevertheless make one pair of strand-exchanges to generate a joint molecule of the Holliday structure type¹⁰. This result rules out recombination models in which the only homology-dependent step is synapsis (the juxtaposing of the two recombination sites). Our results also reveal a functional asymmetry in the recombination sites. We present models for bacteriophage λ integrative recombination which accommodate these findings.

Integration of bacteriophage λ into the chromosome of its *Escherichia coli* host is effected by recombination between the phage and bacterial attachment sites (*attP* and *attB* respectively). Recombination within a 15 bp core sequence common to both attachment sites reassorts the arms to generate two recombinant sites (Fig. 1a). Integration requires two proteins; the phage encoded integrase (Int) protein and integration host factor (IHF) provided by *E. coli*. Int has a topoisomerase activity and thus can mediate breakage and reunion of DNA. Int protein bound to the core-arm junctions can make transient cleavages at specific locations within the cores of *attP* and *attB* (Fig. 1b), and become covalently attached to the DNA. Strand-exchange occurs when the cleaved ends of *attP* are rejoined to the corresponding ends from *attB*. (For reviews see refs 11 and 12). Early evidence from *in vivo* experiments^{13,14} suggested that strand-exchanges occur independently, and the initial event is thus the generation of a Holliday structure¹⁰ (Fig. 1a). Recently such Holliday structures have been detected during *in vitro* recombinations (P.A.K. and H.A.N., manuscript in preparation; A. Landy, personal communication). A second strand-exchange converts the Holliday structure into the complete recombinant products (Fig. 1a). This part of the reaction has also been demonstrated using the *in vitro* recombination system (ref. 15; B. de Massy and R. A. Weisberg, personal communication).

The Int cleavage sites on the two strands of an attachment site are staggered by 7 bp (ref. 11) (Fig. 1b) thereby creating an 'overlap region'. In the products of recombination this region contains one strand from each parental site. Alterations to the overlap sequence result in site-affinity (*saf*) mutants which recombine poorly with a wild-type partner, but recombine at near

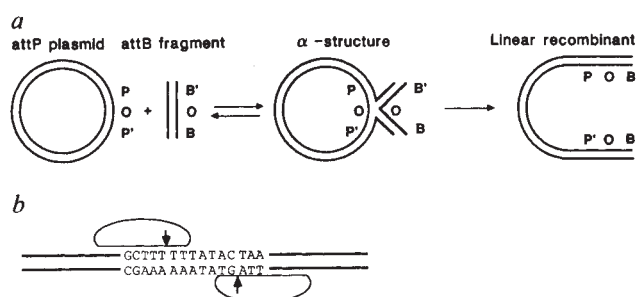


Fig. 1 a, Recombination through a Holliday structure intermediate. The phage attachment site is shown as POP' to represent the common core (O) flanked by the two different arms of *attP* (P and P'). Similarly, *attB* is written BOB'. Recombination of one strand of *attP* with the corresponding strand of *attB* makes a Holliday junction at the attachment sites; with circular *attP* and linear *attB* substrates, this is part of an α -structure. Recombination of the second pair of strands generates the completed product containing the recombinant sites BOP' and POB'. b, Int binding to the core of an attachment site. The 15 bp core sequence of an attachment site is shown with two Int molecules bound at the core-arm junctions¹¹. Arrow, the position at which Int cleaves the strand¹¹. By convention, top and bottom strands, left and right refer to the attachment site as drawn.

normal efficiency with an attachment site carrying the same *saf* mutation¹⁻³. Attachment sites with many different overlap sequences recombine efficiently provided that the two recombining sites are perfectly homologous in this region. This requirement for homology is apparently limited to the overlap region³. Other site-specific recombination reactions (those mediated by the Hin, Cre and FLP recombinases) display a similar dependence on localized homology⁴⁻⁹. The role for homology in these systems could be to allow annealing of the cohesive ends that would be produced by double-strand breaks in the recombination sites. This simple model has, however, been ruled out for phage λ site-specific recombination¹⁶. In this paper we test an alternative model for the role of homology in integrative recombination.

A critical step in recombination is synapsis, the juxtaposing of the recombination sites that must precede the first strand-exchange. A current model for phage λ integrative recombination postulates that synapsis is stabilized by formation of a block of four-stranded DNA (4s-DNA) across the overlap regions^{17,18}. On stereochemical grounds, it is thought that 4s-DNA could only be formed from two DNA duplexes of the same sequence¹⁹. In this model, therefore, homology across the whole overlap region is required to make a stable synapsis and all the subsequent steps are homology-independent. The model predicts that in recombination between attachment sites with different overlap sequences, synapsis will be destabilized and hence that production of Holliday structures and completed recombinant products will be reduced coordinately. We have tested this prediction.

Recombination *in vitro* is inhibited by substitution of thiophosphate for phosphate at either of the Int cleavage sites in *attB* (ref. 20). If exchange of the bottom strands is blocked in this way, the very small amount of Holliday structure intermediates present under standard conditions can be boosted to readily detectable amounts (P.A.K. and H.A.N., manuscript in preparation). In our assay such intermediates take the form of α -structures (Fig. 1a). The formation of α -structures can be further stimulated by the use of a variant Int protein, known as Int-h (refs 21 and 22), and with this Int protein α -structures can also be detected in unblocked recombinations (P.A.K. and H.A.N., in preparation). Recombination with an *attB* containing a nick at one Int cleavage site also yields a high level of α -structures (A. Landy, personal communication). We have used our α -formation assay to examine the requirement for homology in the early steps of recombination.

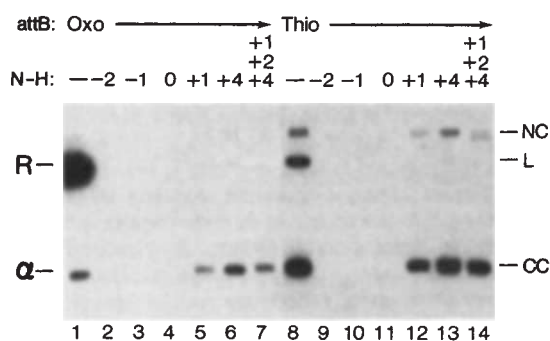


Fig. 2 Effect of non-homology on α -structure formation and recombination. Autoradiograph of an agarose gel showing the products of recombination between a ^{32}P -labelled linear DNA fragment (60 bp) carrying *attB* *saf*⁺ and a supercoiled plasmid (4.5 kilobases (kb)) carrying an *attP* variant as follows: lanes 1 and 8 *saf*⁺, lanes 2 and 9 *saf*^{-2G}, lanes 3 and 10 *saf*^{-1G}, lanes 4 and 11 *saf*^{0C}, lanes 5 and 12 *saf*^{+1G}, lanes 6 and 13 *saf*^T, and lanes 7 and 14 *saf*^G. In lanes 8-14 the *attB* contained thiophosphate at the Int cleavage site in the bottom strand. In lanes 1-7 the *attB* contained all normal (oxo) phosphates. N-H, the position(s) at which the overlap regions of the two recombining sites are non-homologous. NC, L and CC, positions of the nicked circular, linear and covalently closed forms of the *attP* substrate plasmid. The *attB* fragment makes a negligible contribution to the size of the products; therefore, linear recombinants (R) comigrate with the linear form of the *attP* plasmid and α -structures comigrate with the covalently closed plasmid. (Base pairing in the B and B' arms of the α -structures constrains the circular portion of the molecule; the majority of α -structures therefore migrate as if they were covalently closed, although a small fraction run with the nicked circular plasmid). The structure of α -forms like those shown in lanes 1 and 8, has been confirmed by analysis of the component strands after restriction. The *attB* fragment has run off the gel.

Methods. The *attP* substrate plasmids were constructed by recloning the 493 bp *HindIII*-*Bam*HI fragments carrying the *attP* *saf* sites from M13 derivatives into pBR322 using standard procedures²⁶. The *attB* substrates were synthesized by annealing an *attB* top strand template, labelled with ^{32}P at the 5' end, and a primer complementary to the sequences from +5 to +26. This bottom strand primer was extended using DNA polymerase and either all four dNTPs or three dNTPs plus the α -thiophosphate derivative of dGTP. Detailed protocols for the synthesis and purification of the *attB* substrates will be published elsewhere (P.A.K. and H.A.N., in preparation). Integrative recombination reactions (20 μl) contained 50 mM Tris-HCl (pH 8.0), 70 mM KCl, 5 mM spermidine, 1 mM sodium EDTA, 1 mg ml⁻¹ bovine serum albumin (BSA), 4.5 nM of a supercoiled plasmid containing *attP*, 0.25 nM *attB* fragment, 100 nM purified IHF (ref. 27) and 200 nM purified Int-h (ref. 22). After incubation at 25 °C for 2 h reactions were terminated by addition of 5 μl of a solution containing 10% Ficoll, 0.5% SDS and 1 mg ml⁻¹ bromophenol blue, and electrophoresed through 0.7% agarose gels in 1×TBE containing 0.5 μg ml⁻¹ ethidium bromide. The gels were dried and autoradiographed on Kodak XAR film with an intensifying screen at -80 °C.

Figure 2 shows the production of α -structures and linear recombinants from reactions containing a wild-type (*saf*⁺) *attB* and either *attP* *saf*⁺ or one of several different *attP* *saf* variants (Table 1). In homologous recombination reactions both linear recombinants and α -structures are made. In recombination reactions with *attB* sites containing thiophosphate, the second strand exchange is depressed, leading to a reduction in linear recombinants and an increase in α -structures (Fig. 2, lane 8 compared to 1). The effect of non-homology on α -formation is therefore most easily visualized in recombinations with the thiophosphate *attB* (Fig. 2, lanes 8-14). Any non-homology is a strong block to production of linear recombinant; several non-homologous pairings, however, yield levels of α -structure only slightly lower than is seen in an homologous recombination (Fig. 2, lanes 12, 13, 14 compared with lane 8). These results also reveal that non-homology in the left part of the overlap region (at positions

Table 1 The overlap region of *saf* mutants

	-2	0	+2	+4			
<i>saf</i> ⁺	T	T	T	A	T	A	C
<i>saf</i> ^{-2G}	G	.	.	.	.	.	.
<i>saf</i> ^{-2A}	A	.	.	.	.	.	.
<i>saf</i> ^{-1G}	.	G	.	.	.	.	.
<i>saf</i> ^{-1C}	.	C	.	.	.	.	.
<i>saf</i> ^{-1A}	.	.	.	.	.	.	.
<i>saf</i> ^{0C}	.	.	.	.	.	.	.
<i>saf</i> ^{0A}	.	.	.	.	.	.	.
<i>saf</i> ^{+1G}	.	.	.	.	.	.	.
<i>saf</i> ^T	.	.	.	.	.	.	.
<i>saf</i> ^G	.	.	.	.	.	.	.

The sequence of the top strand for the wild-type (*saf*⁺) overlap region is shown in full; sequence for a *saf* mutant is shown only where it differs from wild-type. Coordinates are given with reference to the central T-A base pair of the core, which is defined as position 0 (ref. 11). Mutants *saf*^G, *saf*^T, *saf*^{-2G} and *saf*^{-2A} have been described previously¹⁻³. Mutant *saf*^{+1G} also has the canonical *saf* phenotype (C. E. Bauer and J. F. Gardner, personal communication). The *saf* mutants with changes at the -1 and 0 positions were made for this study by site-directed mutagenesis²⁵. In *in vitro* recombination assays with an *attP* *saf* mutant and *attB* *saf*⁺, all changes examined (-1T to G, C or A; 0T to C or A) reduce recombination drastically (Fig. 2, lanes 3 and 4; data not shown). Recombination between *attP* *saf*^{0C} or *attP* *saf*^{0A} and an *attB* carrying the same mutation occurs with an efficiency similar to that of *saf*⁺-by-*saf*⁺ recombination (data not shown). Recombination between *attP* *saf*^{-1G}, *attP* *saf*^{-1C} or *attP* *saf*^{-1A}, and the homologous *attB*, occurs with a slightly lower efficiency (data not shown), presumably because the T at -1 is a conserved base in the Int consensus binding sequence¹¹.

-2, -1 and 0) has a different effect on recombination than non-homology in the right part (positions +1 and +4). Several other mutants with changes located on the left (*attP* *saf* mutants -2A, -1C, -1A, 0A) also give no detectable products in this assay (data not shown). The recombinations in which the *attB* substrate contains no thiophosphate (Fig. 2, lanes 1-7) confirm that *saf* mutants with changes in the right of the overlap region block recombinant formation more strongly than they affect accumulation of α -structures, and that non-homology on the left prevents accumulation of both products. A similar pattern is also seen in recombinations mediated by wild-type Int protein, although the levels of α -structures are much lower (data not shown). The different consequences of introducing non-homology into the left or the right of the overlap region reveal an asymmetry in the recombination mechanism. Other experiments corroborate this conclusion and imply that strand-exchange on the left (involving top strands) is the primary event (P.A.K. and H.A.N., in preparation; A. Landy, personal communication).

The result that non-homologies on the right of the overlap region have only a small effect on the accumulation of α -structures but are strong blocks to production of completed recombinants, is contrary to the predictions of the 4s-DNA model outlined above. Involvement of homology after the first strand-exchange is indicated because, in several recombinations blocked only by non-homology, α -structures accumulate but are not processed on to linear recombinants (Fig. 2, lanes 5-7). The conversion of synthetic Holliday junctions into recombinant products is also blocked by *saf*^G/*saf*⁺ non-homology (B. de Massy and R. A. Weisberg, personal communication). Recombination models, such as the 4s-DNA model, in which the only homology-dependent step is synapsis are ruled out by these results.

Of many possible models for the role of homology in this system, we have seriously considered two alternatives. The first postulates that the act of strand exchange requires a few homologous base-pairs adjacent to the site of the exchange. Non-homology on the right would permit the attachment sites to come together and exchange the top strands (at the left of the overlap region) to form α -structures, but would block the bottom strand exchange (at the right) that would normally convert the α -forms into linear recombinants. Non-homology on the left would block top strand exchange and since the α -structures that accumulate in unblocked recombinations are all the products of top strand exchange (P.A.K. and H.A.N.,

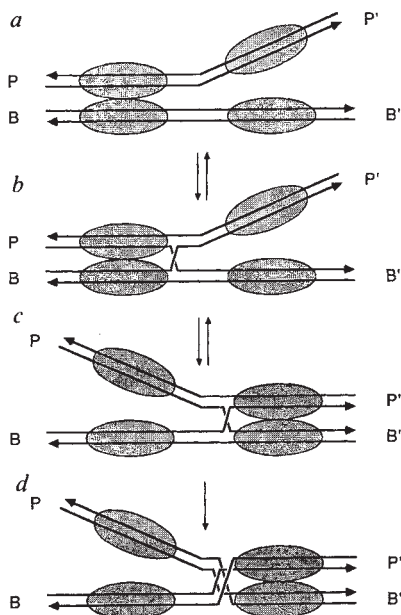


Fig. 3 Discontinuous branch-migration model. Synapsis juxtaposes the two Int protomers bound to the left side of the *attP* and *attB* cores (a). The first strand exchange makes a Holliday junction on the left of the overlap region (b). This strand exchange will be rapidly reversed unless the Holliday junction moves rightward, away from the Int proteins at the left core-arm junctions. (We imagine that Int protomers bound to the cores confine the Holliday junctions to the overlap region.) Movement of the Holliday junction to the right requires a change in the conformation of the synaptic structure which concomitantly moves the Holliday junction three base-pairs to the right and juxtaposes the Int protomers bound to the right core-arm junctions (c). (Although this conformational change is represented by tipping of the structure, similar to the action of a see-saw, in three dimensions the realignment might involve rotation of the DNA helices or isomerization of the Holliday junction.) After this conformational change, the Holliday junction is free to migrate further to the right, or alternatively, the conformational change can be reversed moving the junction back to the left. If the Holliday junction moves to the righthand edge of the overlap region it will prompt the Int protomers bound to the right core-arm junctions to make the second strand exchange (d). This generates the fully recombinant sites which will separate, preventing reversal.

manuscript in preparation), no products would accumulate. An example of a strand-exchange mechanism that would result in a requirement for 'local' homology is one which requires that a three-base segment of donor DNA be Watson-Crick paired with the recipient duplex to permit efficient resealing⁶.

A second model that can explain our results, and the one which we favour, is one in which homology is required to permit a Holliday junction to branch migrate from one side of the overlap region to the other¹. This model is appealing because it readily explains recombination of heteroduplex attachment sites^{3,16}. A version of this model which can account for the asymmetric effect of non-homology on α -formation is shown in Fig. 3. In this model, Holliday junctions are made at the left side of the overlap region but are rapidly resolved back to substrates. They can be partially stabilized by moving away from the Int protomers that made them, into the overlap region, but the minimum first step is three base-pairs. Holliday junctions that reach the other side of the overlap region are converted into fully stable recombinant products. In recombinations blocked by non-homology at the -2, -1 or 0 positions, Holliday junctions formed by top strand exchange will be trapped at the left boundary of the overlap region and will get resolved back to substrates. If all the non-homology lies to the right of the 0 position, Holliday junctions made at the left can move rightward and some of them will persist.

The Cre and FLP site-specific recombination systems have overlap regions similar in size to that of the λ attachment sites^{23,7,24} and a branch-migration model could explain the homology requirement in these systems. General recombination systems might also search for homology by a mechanism analogous to the way we think homology is sensed in phage λ integration. For example, a transient invasion of the recombinogenic strand into the recipient duplex, requiring little or no homology, could create a small D-loop. In a second step, the D-loop could be enlarged by branch-migration; if this homology-dependent step failed, the initial invasion would reverse.

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Note added in proof: Since submission of this paper, an account of the experiments that use nicked *attB* substrates to trap Holliday structures has been prepared (Nunes-Düby, S. E., Matsumoto, L. & Landy, A. *Cell*, in the press). Holliday structure formation by the Cre recombinase has also been reported (Hoess, R., Wierzbicki, A. & Abremski, K. *Proc. natn. Acad. Sci. U.S.A.*, in the press).

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The heat shock response of *E. coli* is regulated by changes in the concentration of σ^{32}

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Cells subjected to a heat shock, or a variety of other stresses, increase the synthesis of a set of proteins, known as heat shock proteins¹⁻³. This response is apparently universal, occurring in the entire range from bacterial to mammalian cells. In *Escherichia coli* heat shock protein synthesis transiently increases following a shift from 30 °C to 42 °C as a result of changes in transcription initiation at heat shock promoters⁴⁻⁶. Heat shock promoters are

recognized by RNA polymerase containing a sigma factor of relative molecular mass (M_r) 32,000 (32K) σ^{32} (refs 7, 8) and not σ^{70} , the major form of RNA polymerase holoenzyme⁶. To determine whether changes in the concentration of σ^{32} regulate this response, we measured the amount of σ^{32} before and after shift to high temperature and found that it increased transiently during heat shock as a result of changes in σ^{32} synthesis and stability. Our results indicate that σ^{32} is directly responsible for regulation of the heat shock response.

The amount of σ^{32} was measured at 30 °C or after shift to 42 °C using Western blot analysis (Fig. 1). At 30 °C σ^{32} was present at less than 50 molecules per cell, as judged by comparison with known amounts of σ^{32} : σ^{70} is present at 3,000 molecules per cell⁹. Following temperature shift, there was a rapid and significant increase in the amount of σ^{32} (Fig. 1a). By five to six min, following a shift from 30 °C to 42 °C, the sigma factor increased ~17-fold (Fig. 1b). By 15 min at 42 °C, the amount of σ^{32} had dropped to only five times the amount at 30 °C. Lesley *et al.*¹⁰ have also observed a transient increase in σ^{32} during heat shock. We compared the increase of σ^{32} with the relative synthesis of messenger RNA from a heat shock operon, the *dnaKdnaJ* operon, following a shift from 30 °C to 42 °C (Fig. 1b). The magnitude and kinetics of the change in σ^{32} concentration are sufficient to account for the changes in *dnaKdnaJ* mRNA synthesis.

Changes in both the synthesis and stability of σ^{32} contribute to the transient increase in σ^{32} during heat shock. Immunoprecipitation analysis showed that σ^{32} synthesis increased 11-fold by 3–4 min after temperature shift, but then dropped to about six times the rate at 30 °C after 6 min at 42 °C (Fig. 2a). In addition, σ^{32} was rapidly degraded during steady state growth at 30 °C or 42 °C, $T_{1/2}$ = 1 min (Fig. 2b), but was at least eight times more stable during the first 4 min after shift to 42 °C (Fig. 2c). The kinetics of the changes in σ^{32} synthesis and stability are consistent with the kinetics of the change in σ^{32} level after temperature shift. The peak of synthesis, and the time of stabilization, occur slightly before the peak accumulation of σ^{32} (Fig. 1b). The rate at which the amount of a protein changes in response to alterations in its synthesis and stability is controlled by the extent of its instability¹¹. It is the extreme instability of σ^{32} at 30 °C that permits the rapid adjustment in its concentration that mediates the heat shock response.

To examine the mechanism regulating σ^{32} synthesis, we compared the behaviour of a protein fusion (pDS4) and an operon fusion (pDS3) between *rpoH*, the gene for σ^{32} , and *lacZ*, the structural gene for β -galactosidase, during a heat shock (Fig. 3). Synthesis of β -galactosidase from the operon fusion is controlled by the transcriptional signals of *rpoH* and translational signals of *lacZ*, and synthesis of the fusion protein is controlled by both the transcriptional and translational signals of *rpoH*. Only the fusion protein showed the transient increase in synthesis characteristic of σ^{32} . This result suggests that the changes in σ^{32} synthesis during heat shock are regulated at the level of translation. Our conclusion is in accord with the observations that there is little increase in *rpoH* mRNA synthesis following a shift to high temperature¹², and that σ^{32} synthesis directed by the λP_L promoter is post-transcriptionally repressed during the shut-off of the heat shock response¹³. The accumulation of *rpoH* mRNA following shift to high temperature^{14,12} follows the increase in synthesis of σ^{32} , and it is therefore likely to be the result of increased message translation. This has been observed for the regulation of ribosomal protein gene expression¹⁵.

The mechanism of the cellular response to ethanol stress is similar to that for heat shock. An increase in the amount of σ^{32} was observed when the heat shock proteins were induced with ethanol (Fig. 4a and b). The extent and duration of the increase in σ^{32} corresponded to the changes in synthesis of heat shock proteins. The changes in the amount of σ^{32} following ethanol addition, as during heat shock, resulted from changes in both the synthesis and stability of σ^{32} (data not shown).

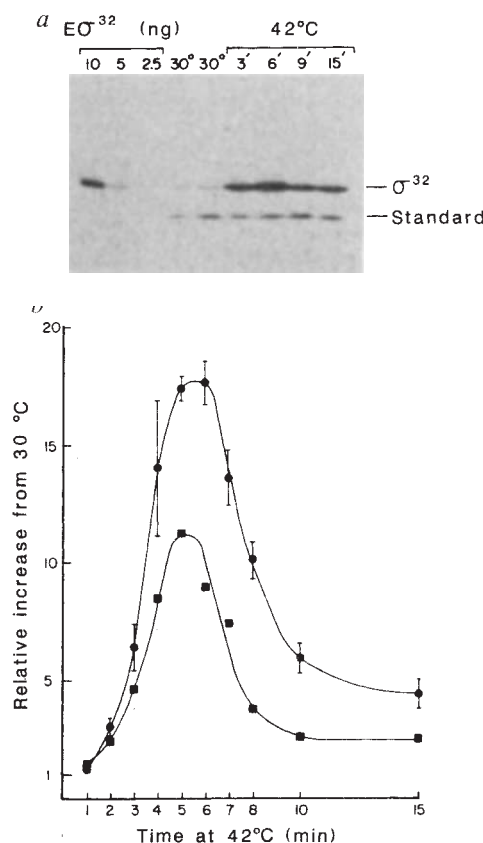


Fig. 1 The level of σ^{32} changes during heat shock. *a*, Western blot of samples of equal volume from cells growing at 30 °C, or at 3, 6, 9 and 15 min after shift to 42 °C (lanes 4–9). Lanes 1–3 are 10, 5 and 2.5 ng of purified σ^{32} . *b*, Quantitation of changes in σ^{32} (●), compiled from five experiments, and relative *dnaKdnaJ* mRNA synthesis (■), following shift from 30 °C to 42 °C.

Methods. Strain C600 (CAG11064) was grown to mid-log phase in M9-glucose, or MOPS-glucose media, supplemented with all amino acids. For analysis of σ^{32} , 1-ml aliquots were sampled into trichloroacetic acid (TCA) (final concentration 5%). To control for losses during the analysis, a constant volume of cell extract from strain CAG11033 (supplied by R. Calender), which overproduces a lower molecular weight form of σ^{32} encoded by *rpoH113* (ref. 22), was added to each sample. The TCA precipitates were resuspended in SDS-sample buffer²³, heated at 100 °C, resolved on 10% SDS-polyacrylamide gels²³, and electrophoretically transferred to nitrocellulose²⁴. Blots were blocked with TBS-1% nonfat-dry milk²⁵, and developed with sequential incubations in dilutions of anti- σ^{32} sera, purified alkaline phosphatase conjugated goat anti-rabbit antibodies (CalBiochem), and 5-bromo-4-chloro-3-indolyl-phosphate with nitroblue tetrazolium²⁶. Rabbit antibodies to the sigma factor were elicited by injection of crushed polyacrylamide gel slices containing σ^{32} (ref. 27) following one-dimensional SDS-PAGE of purified σ^{32} (refs 7, 12). To remove non- σ^{32} specific antibodies, dilutions of σ^{32} antisera were adsorbed with an extract of strain CAG9301, which does not produce σ^{32} as the result of an insertion in the 5' end of the *rpoH* gene (Zhou, Y. N. *et al.*, in preparation). For quantitative analysis samples were diluted before resolution on gels to give approximately the same level of σ^{32} . Blots were scanned with a reflective densitometer and the ratio of σ^{32} to the *rpoH113* σ^{32} standard was determined. These ratios are expressed relative to the value at 30 °C and corrected for sample dilution and a cell doubling time of 40 min following shift to 42 °C. The average values of five experiments, $\pm$ s.e.m., are presented. The synthesis of *dnaKdnaJ* mRNA relative to total RNA synthesis was determined by filter hybridization as described by Erickson *et al.*¹². Aliquots of CAG11064 were pulse-labelled with [³H]uridine for 1 min, RNA extracted and hybridized to λ *dnaKdnaJ* DNA bound to nitrocellulose filters. The fraction of total ³H label that hybridized to the λ *dnaKdnaJ* filter, minus background, is expressed relative to the average of duplicate 30 °C values (86 c.p.m.), minus background (20 c.p.m.).

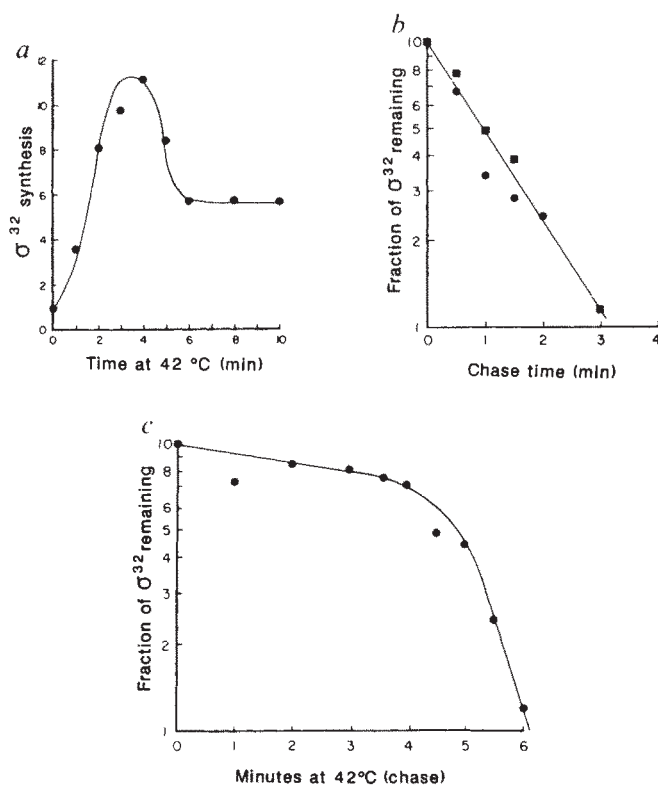


Fig. 2 Synthesis and stability of σ^{32} at 30 °C and after shift to 42 °C. *a*, Synthesis of σ^{32} during heat shock, normalized to the 30 °C rate. *b*, The fraction of radioactive σ^{32} remaining in cultures pulse-labelled during steady state growth at 30 °C (■), or 42 °C (●). Straight line is for $t_{1/2} = 1$ min. *c*, The fraction of radioactive σ^{32} remaining in cultures labelled at 30 °C and then shifted to 42 °C in the presence of excess unlabelled amino acid. Straight-line parts have $t_{1/2}$ (left) 8 min, (right) 1 min.

Methods. Strain CAG11064 was grown to mid-log phase in M9-glucose with all amino acids except methionine. Cultures were labelled with 80 $\mu\text{Ci ml}^{-1}$ [^{35}S] methionine for 1 min. For synthesis experiments samples were removed to TCA immediately following labelling, and for stability experiments excess unlabelled methionine was added after labelling, and 1 ml aliquots removed to TCA at the indicated times. TCA precipitates washed twice with 80% acetone, resuspended in 2% SDS and heated at 100 °C for 3 min. Equal c.p.m. from each sample were immunoprecipitated with anti- σ^{32} antiserum. To control for losses during the analysis equal c.p.m. of strain CAG11033(*rpoH113*), labelled with [^{35}S]methionine, were added to each sample (see legend to Fig. 1). Immunoprecipitations were carried out as described²⁸. Immunoprecipitates were electrophoresed on 10% SDS-polyacrylamide gels, the bands corresponding to σ^{32} and the *rpoH113* σ^{32} standard excised, solubilized, and counted. For the synthesis experiments (*a*) the ratio of σ^{32} to the standard was normalized to the value at 30 °C and corrected for a 2.5-fold increase in the rate of total protein synthesis following shift from 30° to 42° to obtain the change in absolute synthesis of σ^{32} following temperature shift. For the stability experiments (*b*, *c*) the σ^{32} /standard ratio was normalized to the value at 0 min chase.

We have found that a transient increase in the amount of σ^{32} occurs following a shift from 30 °C to 42 °C, and is sufficient to explain the regulation of heat shock protein synthesis. Although the mechanism by which the cell detects a sudden change in temperature remains unknown, σ^{32} has been identified as the acceptor of this sensory information. These results indicate that bacterial σ factors can be directly responsible for controlling gene expression.

Changes in the concentration of σ^{32} are likely to have a general function in the induction of heat shock proteins in response to stress. The close correlation between changes in the amount of σ^{32} and the rate of heat shock gene mRNA synthesis after

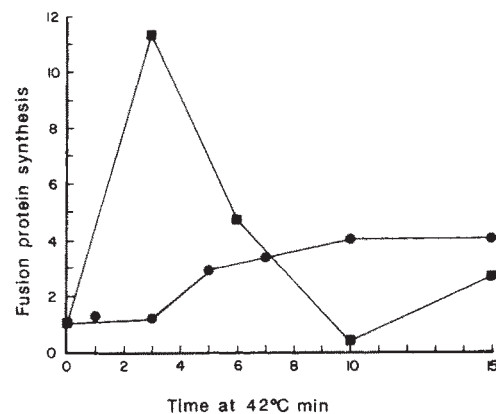


Fig. 3 Synthesis of *rpoH-lacZ* protein fusion and operon fusion gene products during heat shock. The synthesis of the protein fusion (■), and the operon fusion (●), were determined at 30 °C and at various times after shift to 42 °C.

Methods. Strain CAG2041 (CSH26 F'*lacI*^q *lacZ* :: Tn5) carrying plasmid pDS3 (operon fusion: *rpoH'-lacZ*⁺) or pDS4 (protein fusion: *rpoH'-lacZ'*) was grown at 30 °C in M9-glucose medium with ampicillin and all amino acids except leucine and lysine. Aliquots (1 ml) were pulse-labelled with [^3H]leucine and [^3H]lysine for 1 min and transferred into TCA. The same strains labelled with [^{35}S]methionine served as standards for quantitation. The labelled proteins were separated by SDS-PAGE, the fusion products were excised, solubilized, and counted in Aquasure (Amersham) scintillation fluid. Synthesis rates were determined by the double label method¹³. The rates were normalized to the 30 °C values and corrected for a 2.5-fold increase in total protein synthesis following shift from 30 °C to 42 °C to estimate the change in absolute synthesis. Plasmids were constructed by inserting a 3-kilobase (kb) *PvuII* restriction fragment from plasmid pKP11 (ref. 29) into the *SmaI* site of pRS415 (ref. 30) to create pDS3, or the *SmaI* site of pMC1843 (ref. 31) to create pDS4. This restriction fragment carries 800 base pairs (bp) of the *rpoH* gene and all of the promoter region¹². In plasmid pDS3 the restriction fragment is cloned just upstream of the intact *lacZ* gene including the *lacZ* translation initiation region, but without the *lac* promoter. In this construction translation from *rpoH* terminates upstream of *lacZ*. Plasmid pDS4 fuses the *rpoH* coding region in-frame to the eighth codon of the *lacZ* gene.

temperature upshift strongly indicates that both the turn-on and shut-off of the heat shock response are regulated by changes in the intracellular concentration of σ^{32} . Following induction with ethanol, the amount of σ^{32} also increased coordinately with the synthesis of heat shock proteins. The changes in the amount of σ^{32} after temperature shift or addition of ethanol result from changes in both σ^{32} synthesis and stability, suggesting that both stresses generate the same intracellular signal. The induction of heat shock protein synthesis following infection of *E. coli* by bacteriophage λ (refs 16 and 17) is apparently also mediated by changes in the concentration of σ^{32} . The λ cII protein stabilizes overproduced σ^{32} , and presumably induces the heat shock response by causing an increase in the amount of σ^{32} (ref. 18).

The changes in σ^{32} stability during heat shock are consistent with models that suggest that cells detect stressful conditions by the increased presence of unstable polypeptides¹⁹⁻²¹. σ^{32} , the direct effector of the heat shock response, is transiently stabilized following temperature shift, as would be predicted if the proteases responsible for the degradation of σ^{32} were saturated by competing unstable proteins. It is not known, however, whether unstable polypeptides are generated by a heat shock. Alternatively, the brief stabilization might result from a regulatory event specific to σ^{32} , such as reversible modification of σ^{32} , or of a protease which degrades it. As the transient stabilization of σ^{32} does not require its *de novo* synthesis (Fig. 2c), any modifications affecting its activity as a proteolytic substrate must occur post-translationally.

We would like to thank Marilyn Edwards for technical assist-

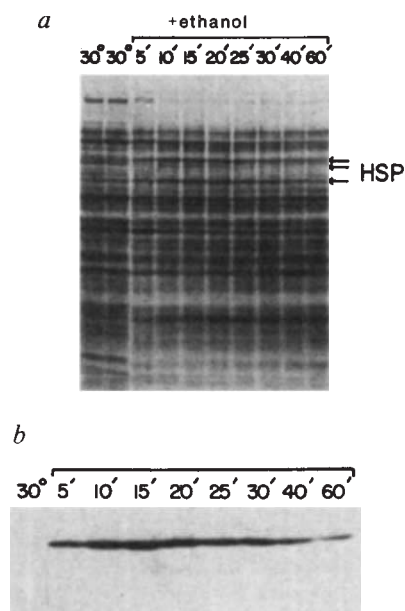


Fig. 4 Heat shock protein synthesis and σ^{32} level following the addition of ethanol. *a*, Protein synthesis before (lane 1), and at indicated times after (lanes 2–8), addition of ethanol. *b*, Western blot analysis of σ^{32} level before and after addition of ethanol as indicated.

Methods. Strain CAG11064 was grown to mid log phase at 30 °C in M9-glucose medium with all amino acids except methionine. Ethanol was added to a final concentration of 4%. Protein synthesis was analysed by pulse-labelling with [35 S] methionine as described¹³. The arrows indicate, in order of increasing mobility, the DnaK, C62.5, and GroEL heat shock proteins. Western blots were performed as described in Fig. 1 legend.

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A structural model for the retroviral proteases

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In many retroviruses the 5' end of the *pol* gene codes for a protease vital for the processing of the *gag* polyprotein into the separate core proteins^{1,2}. In some viruses this protease is encoded at the 3' end of the *gag* gene³, or between the *gag* and *pol* genes in a different reading frame to either⁴. A sequence, Asp-Thr-Gly, which is conserved in retroviral proteases is also conserved in the active sites of aspartic proteases, an observation which has led to the suggestion that the retroviral proteases could belong to this family^{5,6}. We have examined the sequences of the aspartic and retroviral protease families, using pattern-recognition, structure prediction and molecular modelling techniques, and conclude that the viral protease sequences probably correspond to a single domain of an aspartic protease and may function in a dimeric form. We have constructed a model of the *pol*-protease of human immunodeficiency virus 1 (HIV-1) to test this hypothesis.

The known aspartic proteases are two-domain proteins, more than 300 residues in length, with a conserved Asp-Thr-Gly sequence in each domain contributing to the active site. Similarity in sequence and structure between the two domains has led to suggestions of evolution from a single domain by a process of gene duplication and fusion⁷. By contrast, the retroviral sequences, as judged from those for which the N- and C-termini have been determined^{4,8}, do not exceed 130 amino acids in length and contain only one Asp-Thr-Gly sequence. Thus, the retroviral protease sequences can only correspond to a single domain of the aspartic proteases. We have therefore analysed the sequences of both aspartic protease domains to establish the features essential to their common fold and compared these with the viral sequences.

Between proteins that have similar enzymatic function but are otherwise remotely related, the residues involved in catalysis are highly conserved⁹. Other conserved residues are those that from the hydrophobic core and often those with unique stereochemical properties (Gly, Pro, Cys)^{10,11}, both of which can contribute to the conservation of the overall topology or fold to bring the catalytic residues into the correct juxtaposition. The correspondence of such partially conserved sequences is generally too low to allow alignment of distantly related sequences by standard algorithms. We have established a sequence alignment for the retroviral and aspartic proteases based on matching patterns of residue conservation and predictions of secondary structure¹², which allows a rough correspondence to be established where no definite patterns exist. Patterns of conservation in an initial alignment of a family of sequences are used to determine 'consensus templates', which are then iteratively refined until they consistently relocate the sequence from which they arise. The initial alignment for the aspartic proteases was based upon equivalence to the observed crystal structures of endothiapepsin¹³ and penicillopepsin¹⁴ and was established for the retroviral proteases by a conventional alignment method¹⁵. Consistent templates were established for both families of proteases and tested for specificity by matching against 3,800 sequences in the PIR protein sequence database¹⁶. Both templates recognized only proteins belonging to the family from which they were derived and may thus be considered to

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Fig. 1 legend continued: The consensus observed structure for both domains is on line Sa and the corresponding consensus prediction is on line Pa. The predicted secondary structure for the viral sequences is on line Pv. In the predictions and consensus observed structure, the height of the blocks indicates the relative strength of the prediction or consensus. Lines Ta and Tv are templates for aspartic and viral proteases respectively, with the joint template on line Tj. In these templates, upper case letters are absolutely conserved amino acid codes: ~ indicates residues normally associated with β -turns, ● and * indicate strong and preferred hydrophobic positions respectively. 's' is small and hydrophilic, — is a necessary residue with no clear pattern of conservation and Δ is a deletable position. (For an explanation of template matching, see refs 11 and 49.) In the first pattern of the aspartic protease template, ϕ represents a conserved aromatic residue corresponding to a conserved proline in the viral template. In the viral and joint active site templates, 't' signifies threonine or serine. The following pattern (flap) contains positions (g) that indicate a glycine rich turn. In the final viral pattern, 'd' (in GRd) is always hydrophilic and generally charged. The fifth pattern (**_***) is not specific and acts mainly as a 'spacer' for the adjacent patterns, thus within its range only intra-domain alignments are indicated. Details of all the above patterns will be discussed fully elsewhere.

have encapsulated the essential features of the sequences of each family.

Figure 1 shows the aligned sequences of the aspartic and retroviral proteases with their consensus templates. There are other features common to both families apart from the conserved Asp-Thr(Ser)-Gly, notably the absolute conservation of a glycine preceded by two hydrophobic residues. In the aspartic proteases this pattern occurs towards the C-terminus, ~100 residues from the Asp-Thr-Gly pattern, but the two sequences lie beside each other in the tertiary structure in the protein core at the active site. Another pattern, which is roughly conserved between families, corresponds to the highly flexible β -hairpin 'flap' of the N-terminal domains that overlies the active site of aspartic proteases. In view of the strong similarity between the templates for the two families, we have developed a joint template representing only features common to both families (Fig. 1, middle template); this was similarly refined to consistency and found to be unique when tested against the PIR database.

We have also produced joint secondary structure predictions¹² for each family by addition of the individual secondary structure 'probabilities' at each position of the alignment. Comparison of these predicted secondary structures with those actually observed in the crystals of penicillopepsin¹⁴ and endothiapepsin¹³ shows a close correspondence, lending confidence to the secondary structure predicted for the retroviral sequences which are clearly a similar (all- β) type of protein. As we found in the case of the conserved patterns, there is a clear equivalence of predicted structure between the viral and aspartic proteases.

Both these types of analysis imply strong similarities between retroviral proteases and single aspartic protease domains. Although the retroviral proteases as a whole are shorter than an aspartic protease domain by 20 or more residues, most of this deficit occurs in sequences corresponding to the loops connecting the strands of β -sheet that form the core. A further shortening is found at the C-terminus where some of the retroviral sequences (for which C-termini have been determined) appear to lack the final strand seen in the aspartic protease domains. To test whether an aspartic protease domain fold could result from the retroviral sequences, a three-dimensional model was constructed. This was based on the main-chain structure of endothiapepsin (using the molecular modelling program FRODO¹⁷), in which the loops were rebuilt to correspond to the lengths predicted for the *pol*-protease of the AIDS (acquired immune deficiency syndrome) virus HIV-1. As well as being of considerable interest for its importance to the AIDS virus, it is one of the shortest viral sequences, and thus provides the most severe test of our hypothesis.

The modelled HIV-1 *pol*-protease fold is a parsimonious version of that observed in the domains of the aspartic proteases

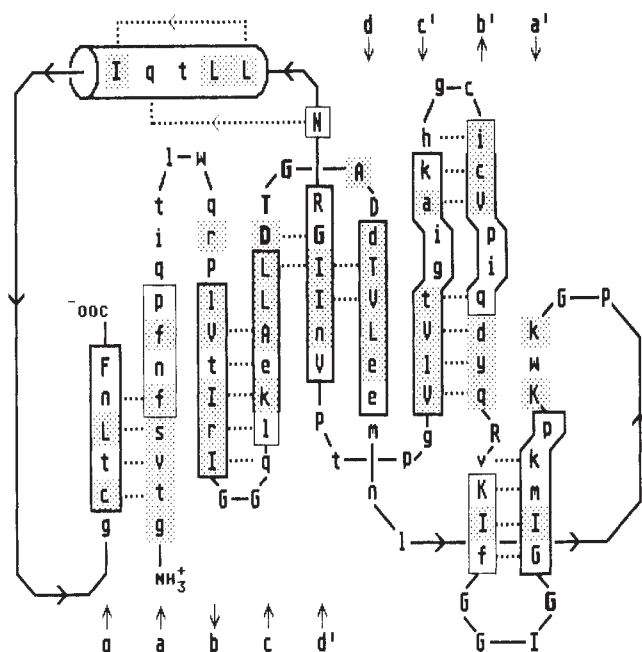


Fig. 2 Schematic diagram of the predicted HIV-1 *pol* protease fold. The amino-acid sequence is given as single letter codes, with lower case indicating residues specific to HIV-1, upper-case indicating residues conserved in retroviral proteases, and bold upper-case indicating residues also conserved in aspartic proteases. Boxes are predicted β -strands, with strong predictions indicated by heavy walls. A predicted α -helix is shown as a cylinder. Residues with a low solvent accessibility have a speckled background.

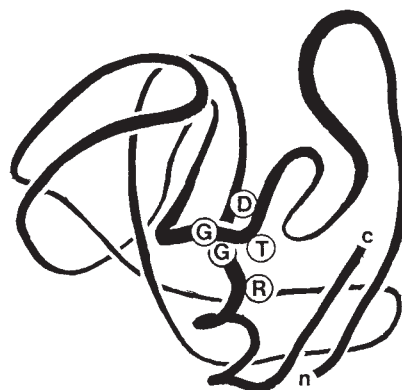


Fig. 3 Predicted tertiary structure of HIV-1 *pol* protease. Diagram of the *pol* protease fold, showing the positions of the residues most highly conserved in the viral sequences.

(Fig. 2). The a-b loop and the c-d loop (which forms the heart of the active site) are the same length as their counterparts in both aspartic protease domains, while the a'-b' loop is intermediate in length between the short C-terminal loop and the longer N-terminal loop which forms the 'flap'. Other loops are generally shorter in the viral fold than in either domain of aspartic proteases, but connect all the β -strands with no disruption of the protein core. Neither the length nor structure of these loops is conserved between domains in the aspartic proteases themselves. The only topological change in the viral fold is the replacement of the C-terminal β -hairpin on the edge of the sheet by a single strand, giving a parallel β -sheet between strands a and q (Fig. 3).

Although the similarity between the two domains of aspartic proteases is restricted to the overall fold and some general sequence conservation, a virtually perfect twofold axis relates the Asp-Thr-Gly residues from each domain in the active site¹⁸.



Fig. 4 Active dimer of HIV-1 *pol* protease. The dimer of the predicted tertiary structure forms the two-fold symmetric active site observed in aspartic proteases¹⁸, and generates the substrate binding cleft. Interactions of the substrate with the enzyme residues lining the binding cleft allow the retroviral proteases to selectively cleave specific X-Pro peptide bonds in the *gag* and *pol* polyproteins⁵⁰.

The dimeric juxtaposition of the aspartate residues serves to bind a water molecule which could be responsible for the enzyme's hydrolytic activity¹⁹. The threonine residues hold the two halves of the active site together in a dimeric side-chain to main-chain interaction. (The only departure from Asp-Thr-Gly among the viral sequences is to Asp-Ser-Gly; serine is the only other amino-acid with the alcohol group required to maintain this interaction.) A single domain protein having only one Asp-Thr-Gly sequence capable of dimerising to produce these essential interactions could act as a competent aspartic protease. We suggest that the retroviral enzymes may be dimeric aspartic proteases, and could perhaps be 'fossil' examples of an ancestral dimeric protease from which the aspartic proteases have evolved.

Using the active site twofold axis from endothiapepsin¹⁸, we have generated a dimer of the three-dimensional model of the HIV-1 protease described above (Fig. 4). As well as providing the required active site structure, the dimer generates a distinct substrate binding cleft, capable of accommodating four to six residues of a substrate peptide. This accords well with the general pattern of cleavage specificity in the *gag*-polyprotein substrates of these enzymes²⁰.

Fully detailed models have been built for aspartic proteases with extensive sequence homology to known crystal structures²¹. Although it would be unwise to perform a comparably detailed operation for the retroviral proteases, the general model presented here has implications which can be tested without a structure determination and will be useful in the design of specific inhibitors of the HIV-1 *pol*-protease and therefore of possible clinical value in the treatment of AIDS.

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Sliding-layer conformational change limited by the quaternary structure of plant RuBisCO

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RuBisCO, D-ribulose-1,5-bisphosphate carboxylase-oxygenase (EC 4.1.1.39), converts carbon dioxide to sugar in the first step of photosynthesis^{1,2}. In plants and some bacteria, this enzyme has an L_8S_8 structure, where L is the large catalytic subunit and S is the small subunit of unknown function. The molecule resembles a keg 105 Å along the 4-fold axis and 132 Å in diameter at the widest point of the keg³⁻⁶. Here we describe the quaternary structure of RuBisCO from *N. tabacum*, the first L_8S_8 type known from an X-ray crystallographic study at near-atomic resolution (3 Å). The structure shows that all eight L subunits are elongated along the 4-fold axis so that the molecule cannot be simply described as layers of subunits, as it had been from studies by electron microscopy^{5,6}. The structure, with its elongated and interdigitated L subunits, is evidence against a large, sliding-layer conformational change in plant RuBisCO, as proposed recently in *Nature* for the same enzyme from *Alcaligenes eutrophus*⁷.

The importance of RuBisCO is not only that it is the source of all food through its catalysis of the initial step of the Calvin cycle of photosynthesis, but also that it is a tempting target for protein engineering because it also catalyses the first step in the

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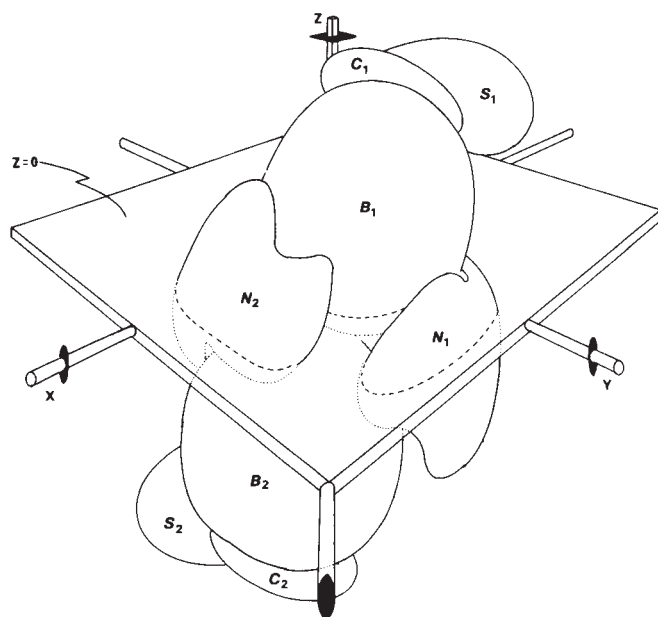


Fig. 1 A schematic sketch of two protomers (labelled 1 and 2) of tobacco RuBisCO in form-III crystals, to define axes and directions. The two protomers are related by the 2-fold axis that bisects x and y . Each large (L) subunit contains three domains: N-terminal (N), barrel (B), and C-terminal (C); S is the small subunit. The 4-fold axis is along z .

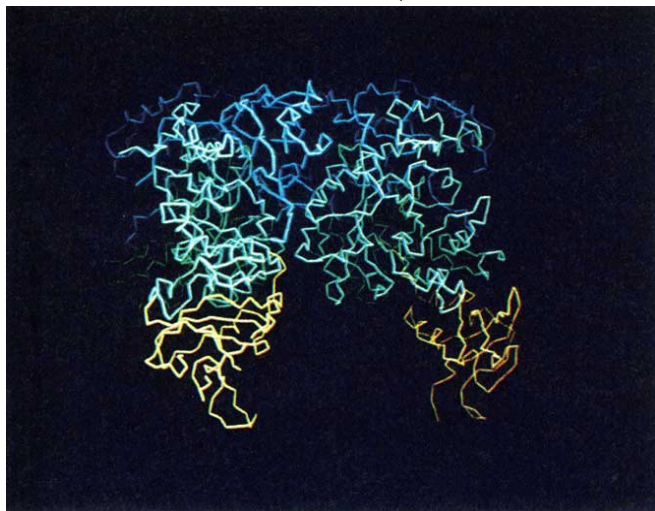


Fig. 2 A partial RuBisCO molecule viewed down the x -axis (see Fig. 1), with the 4-fold (z) axis vertical at the centre of the molecule. The polypeptide backbone is represented here and in Fig. 3 as lines between successive α -carbon atoms. Four S subunits are shown in blue at the top. Two L subunits are in the foreground, with B (barrel) domains in green and N domains in yellow. The small C domain of the L subunit is also in green at the top of the L subunits. The circular opening of the α/β -barrel is visible in the B domain on the right. Notice that the S subunits are bound in a crevice between two sets of B-C domains belonging to two adjacent L subunits. The C-domain extends almost as high along z as the S subunits, so that the four S subunits do not really constitute a separate layer on top.

competing pathway of photorespiration that limits crop yields. Its importance is reflected in structural studies in several laboratories³⁻¹⁵. One recent study has yielded a model for the folding of the polypeptide backbone for the L₂ RuBisCO of the photosynthetic bacterium *Rhodospirillum rubrum*¹⁵, and another has proposed a sliding-layer conformational change in RuBisCO from *A. eutrophus* as it binds the transition-state analogue 2-carboxy-D-arabinitol-1,5-bisphosphate (CABP). This change,

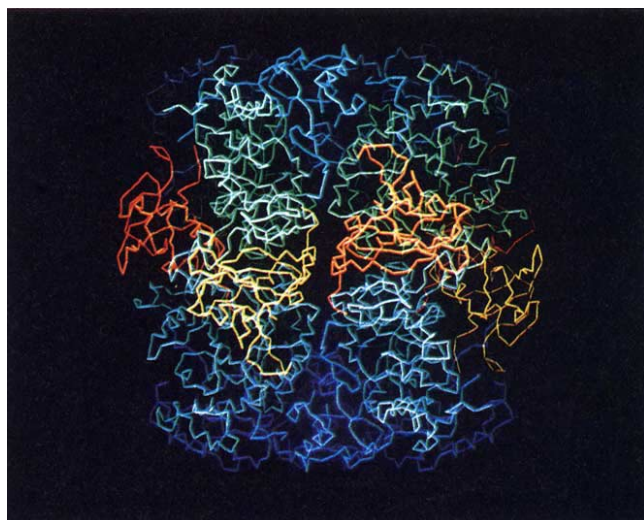


Fig. 3 The entire RuBisCO molecule, viewed in the same direction as in Fig. 2. Tetramers of S subunits are shown in blue, both at high and low z (top and bottom). The L subunits from Fig. 2 are also shown, but they are partly obscured by N domains from other L subunits. The added L subunits have turquoise B domains (in the lower half of the molecule) and have red N domains (in the upper half of the molecule). By comparison with Fig. 2, note that the L subunits cross the central $z = 0$ plane and interdigitate.

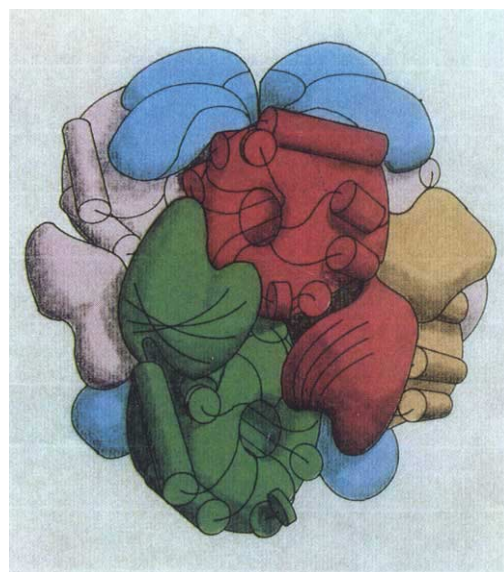


Fig. 4 A schematic drawing of RuBisCO to illustrate the spatial arrangement of subunits and domains. S subunits are in blue, and each L subunit is in a different colour. The view is in the same direction as in Fig. 1, roughly a 45° rotation about the z -axis from the direction of view in Figs 2 and 3. The N-terminal domain of the green L subunit is close to the mouth of the barrel of the red B domain. The small helical C domains are omitted for clarity.

inferred from low resolution data, is a translation perpendicular to the 4-fold axis by 36 Å of one L₄S₄ layer relative to the L₄S₄ layer below.

Our X-ray diffraction study is on form-III crystals of RuBisCO from *Nicotiana tabacum*⁸ at 3-Å resolution. The crystals are grown at high salt and low pH in the absence of CO₂ and Mg²⁺, so that they contain the enzyme in the inactive state. The space group is I422 with one LS pair in the asymmetric unit and with

unit-cell dimensions of $a = 148.7 \text{ \AA}$; $c = 137.5 \text{ \AA}$. Initial X-ray phase determination was by multiple isomorphous replacement (MIR), using three acceptable heavy atom adducts that gave a figure of merit of 0.39 for data to $3.1 \text{ \AA}$ resolution⁴.

Interpretation of this MIR electron density was facilitated by solvent flattening and later through the use of phases calculated from a preliminary model. Solvent flattening¹⁶ was carried out with a reciprocal space algorithm¹⁷, using both automatic and manual definition of solvent regions in the crystal. This process changed the phases by 39 degrees, and produced a map clear enough to permit building of a fragmentary polyalanine model. Following the addition of side chain atoms to fill obvious electron density, this model (model 1) represented about 3,600 of the 4,700 non-hydrogen atoms. Model 1 was refined by a modification¹⁸ of the restrained least-squares method¹⁹. The R -factor to $3.2 \text{ \AA}$ dropped from 48% to 31% for the refined model. Model 2 was built with the aid of electron density maps designed to minimize²⁰ and eliminate²¹ bias from the model. In the largest domain, fragments of model 1 were connected with the topology of the α/β -barrels of pyruvate kinase²² and triosephosphate isomerase²³. The next largest domain could be connected in a fashion similar to the α -carbon diagram of the N-terminal domain of RuBisCO from *R. rubrum*¹⁵. Model 2 contains 4,000 non-hydrogen atoms, in roughly 570 of the 600 residues. Side chains known from the amino-acid sequence^{24,25} have been built into part of the α/β -barrel domain. Model 2 is being refined, the current R factor being 33% at $3.2 \text{ \AA}$ resolution for the 9,700 reflections at least two standard deviations above background noise.

In our model, the L subunit (477 residues; relative molecular mass $M_r = 52,935$) is made up of three domains and resembles the L subunit in RuBisCO from *R. rubrum*¹⁵. The largest domain is the α/β -barrel (B) domain; the next largest is the N terminal (N) domain of about 150 residues, which is largely antiparallel β -sheet with two α -helices along the inner side; the third is a small clump of α -helices at the C-terminus (C domain). The connecting chain between the N domain and the B domain contains two α -helices, both in the tobacco L subunit and in the *R. rubrum* enzyme¹⁵. Moreover, the overall shape and fold of the L subunit in tobacco RuBisCO subunit are similar to those of the L subunit in the L_2 RuBisCO from *R. rubrum*¹⁵. This had been anticipated from the significant cross-correlation of hydrophobicities of the two amino-acid sequences¹³. Another similarity is that our partial fitting of amino-acid sequence in model 2 indicates that the lysine-containing, active site peptides²⁶ are at the C-terminus of β -strands in the B domain, as in *R. rubrum*. Also, a pair of L subunits in tobacco RuBisCO resemble closely the pair of L subunits that make up the *R. rubrum* molecule (Fig. 1). Schneider *et al.*¹⁵ give the dimensions of the L_2 enzyme as about $50 \times 70 \times 105 \text{ \AA}$. The L_2 in the L_8S_8 tobacco RuBisCO has dimensions of $50 \times 68 \times 104 \text{ \AA}$. In short, pairs of L subunits of tobacco RuBisCO are structurally similar to the L_2 RuBisCO of *R. rubrum*.

The structure of tobacco RuBisCO can be generated by tetramerization of *R. rubrum*-like L_2 dimers and addition of the eight S subunits (each 123 residues; $M_r = 14,877$). The quaternary structure of L_8S_8 RuBisCO is illustrated in Figs 2–4. Figure 2 shows a partial RuBisCO molecule, having four S subunits linked to two L subunits in the foreground. The four S subunits are clustered around the 4-fold axis at high z -value. Each small subunit contains β -structure and one prominent α -helix. Each L subunit is elongated along the 4-fold axis, and lies between a pair of S subunits at the top. The S subunits make closest contact with the B and C domains of the L subunits. Figure 3 shows the complete RuBisCO model, viewed from the same direction as in Fig. 2. Clusters of four S subunits are both at the top and bottom, and eight extended L subunits bridge between. A schematic drawing of the molecule is given in Fig. 4. Notice that the elongated L subunits cross over the central $z = 0$ plane of the molecule. This is true for all eight L subunits.

The quaternary structure has implications for the assembly and function of plant RuBisCOs. (1) The RuBisCO molecule cannot be described as a simple two- or four-layered structure as it was in earlier models based on electron microscopy^{5–7}. Instead, all eight L subunits cross the $z = 0$ plane so that there is a top-to-bottom covalent link between the N domain and the B domain of each L subunit. In addition, there are extensive top-to-bottom non-covalent contacts between B domains on different L subunits and between the N domain of one subunit and the B domain of another (Fig. 1). Also inconsistent with description of the molecule as a layered structure, is our finding that the tetramers of S subunits are nestled among four B–C domains. (2) Because of this top-to-bottom covalent linkage and of L subunits, a large conformational change by translation of layers seems implausible. The proposed translation⁷ by $36 \text{ \AA}$ of the upper half of the L_8S_8 RuBisCO of *A. eutrophus* relative to the lower half, as the enzyme binds CABP, could occur in plant RuBisCOs only by unprecedented, large-scale polypeptide unfolding. Our results do not rule out small conformational changes involving motions of a few ångströms as CABP is bound, but they are inconsistent with large scale, sliding-layer conformational changes. (3) The active-site region is at the interface of two L subunits. Part of the N domain is near the entrance of the active-site barrel of the B domain of the adjacent L subunit. This N domain could be involved in regulation, or the catalytic site may actually involve residues both on the B and N domains. In fact affinity labelling of *R. rubrum* RuBisCO shows that the active site is formed from residues from portions of the polypeptide chain that are now known to be in both the N and B domains²⁷. (4) Although the function of the S subunits remains uncertain, the quaternary structure, with two clusters of four S subunits, suggests that tetramers of S subunits may act as a scaffold for L subunits in the assembly of the molecule. We note that tetramers of S subunits at the extremities of the molecule were inferred⁵ from electron micrographs of RuBisCO from *A. eutrophus*. Contiguous small subunits were also correctly suggested²⁸ on the basis of chemical cross-linking. Our observation that the S subunit is not near the mouth of the barrel of the catalytic B domain is consistent with the general agreement¹ that the S subunits are not directly involved in catalysis. However, on the basis of their positions in the structure, it is conceivable that S subunits could mediate any communication between pairs of active-site B domains.

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The invisible made visible

H.V. Wyatt

Research newsletters may be the untraceable publishing the uncitable. But they help keep the wheels of biological science turning.

If there were no Neurospora Newsletter, it would be like a day without sunshine. University geneticist.

NOMENCLATURE, mapping genes, distribution of animals and plants, bibliographies and minor methods are the nuts and bolts of biological research but are unpublishable in the journals. In the early 1930s, however, geneticists hit upon a cheap and efficient method of circulating the data within their invisible colleges. The informal news-sheets *Maize Newsletter* (1932) and *Drosophila Information Service* or *DIS* (1934) contained short technical notes, research reports, information on gene loci and other such material. These news-sheets have grown and prospered¹.

DIS is sent to more than 1,300 subscribers. A recent issue of 150 pages included 117 research notes with graphs and tables, 24 technical and 3 teaching notes. A bibliography contained 2,143 items submitted by recipients. A further bibliography of the Hawaiian *Drosophilida* listed 400 items from 1900 to 1982. There were also 30 pages of information about new mutants from 11 laboratories and a page of information about collections in Italy, India, Peru, Spain, Britain and the United States. The editor described *DIS* to me as "already a research journal by size and content" (personal communication) — yet a journal is what it is *not*. On the cover a box proclaims: "Material presented here should not be used in publications without consent of the author". *DIS* is not a journal but a research newsletter.

Private

Research newsletters are in the private not the public domain. They are found in very few libraries but in many departmental tearooms and laboratories. They are the invisible colleges made visible, for research newsletters are about communication and cooperation. They are an extension of the open laboratory of T.H. Morgan of Columbia University and an expression of the research school which he founded. Citation is usually camouflaged as "personal communication" but research newsletters are an important and growing form of cheap communication beyond an immediate circle.

The first research newsletters were in genetics, but in the early 1950s some zoology ones started, for example *Eaton*

(1954–), and in the 1960s there were several based on computer taxonomy, for example *Taxonometrics* (1962–1970). There are now more than 100 of them, the greatest growth being in zoology. There are about 30 in plant genetics, 14 in animal and 12 in bacterial genetics, 5 in tissue culture, 50 in zoology and 20 in computing in the life sciences and miscellaneous. All are based on a specific organism or group, or a specific research topic.

One reason why research newsletters are born is because scientists in a particular area find they facilitate cooperation. For example, *FRASS* was formed to provide a forum for insect rearing — in 1975 "journals were categorically rejecting manuscripts on the subject. Quality control was an almost non-existent practice. Obsolescence and duplication were rampant". Through *FRASS*, projects and workshops were organized and booklets published. Insect rearing is now recognized as an important activity but *FRASS* remains the primary source of current information. *Clubroot Newsletter* is the focus of cooperative trials of seeds; *Laboratory Primate Newsletter* conducts quarterly surveys of deaths of animals and morbidity data by species; *Trichogramma News* has a working group to initiate and coordinate research. The two editors of *Carnivore Genetics Newsletter* are "convinced that the many investigations by numerous workers worldwide upon cat population genetics would not have occurred but for the stimulus provided by the newsletter".

Items for research newsletters are not refereed or edited (except occasionally for grammar): the editor is more compiler and manager. Because the size of the newsletter is related to the number in the invisible college, the cost is stable at a few dollars a year regardless of numbers. Some consist of a few pages, appearing irregularly as material is submitted, with as few as 50 copies. Some are subsidized by an institution which pays for the photocopying or for the stencils and postage. Contrary to popular belief, research newsletters do not grow up to be journals — only one has changed to the public domain — but continuing the volume numbers. Newsletters arise because of a need and when the need stops, so does the newsletter.

They can be fun, as implied by *Leishmaniac* and *Primate Eye*, and their contents can be varied. *FABIS* carries recipes

— for the kitchen not the laboratory — using faba beans, as fillers between articles. But for the most part the contents are serious. Three services are particularly useful and are seldom given space elsewhere.

The first is the provision of bibliographies — most research newsletters carry a listing of recent work. In a few, it consists of a printout from a computer search of a major abstract or index database, or literature culled from a library. Most, however, carry a unique type of bibliography supplied by the members of the newsletter sending in lists of their publications. The resulting bibliography is very complete, including reports, theses and papers in obscure journals. *Zoological Record* for 1980 did not contain a single new species not already noted in *Amphipod Newsletter*, although a number of new species noted in the latter had been missed by *Zoological Record*.

Scope

In 1967 I compared the scope of the bibliography of *DIS* with *Biological Abstracts (BA)*: 23 per cent of the journals in *DIS* were not scanned by *BA* which would have missed 11 per cent of the 392 papers². The annual number of references in *DIS* has grown from 500 in 1962 to 2,143 in 1982. Of the 2,143, about 35 per cent were abstracts and 20 per cent from *DIS* itself, leaving about 900 papers and books which might appear in *BA*. Of these about 800 contained *Drosophila* or other species in the title and should be assigned to the Generic Index. The Generic Index and Major Concept Index of *BA* for 1982 yielded only about 550 references. A very lengthy and boring manual search of *BA* would yield only about 70 per cent of the journal references so conveniently listed in *DIS*¹. In the case of zoology newsletters, the efficiency may be very much greater — *Symphytos* had recent papers published in Chinese, Indonesian, Hungarian, Russian, Japanese, Italian and Polish, as well as in journals published in Thailand, Lithuania, Yugoslavia, East Germany and Czechoslovakia.

Newsletters often list papers accepted and in press, thus giving notice long before the papers appear in the secondary serials². For the Third World, where library facilities are often meagre and there are considerable postal delays³, newsletter bibliographies must be a wonderful resource. Several of the newsletter bibliographies have been collected and published in hardback.

The second service is publication of listings of culture and stock collections. The geneticist requires access to living mutants and strains, and it is not surprising that one of the first functions of the genetic newsletters was to promulgate details of the availability of such stocks. At least

nine newsletters are linked with stock centres and others may be more loosely connected. Through *DIS*, there are two stock centres in the United States and one in Sweden. A recent list contained over 4,500 items. The stock centre for species other than *D. melanogaster* had a list of 370 species named and unnamed and 2,920 individual items.

Collections

Finally, there is the museum collection; for the zoologist or botanist, this is the equivalent of the geneticist's stock collection. Collections are scattered like unregarded chaff over the world¹ — Manchester University's database records more than 18,000 natural science collections in Britain alone. *Ichnews* has a section on museum collections; *Circulio* published an account of the collections in Hamburg University Museum; *Tymbal* recorded that Carolina State University has 250,000 specimens of Homoptera and that the university library has important literature holdings. *Heteropterists' Newsletter* evaluated 115 collections from 35 countries. *Coccidologist's Newsletter* had a 29-page list of species at Beltsville, including 1,400 species, 60,000 slides and over 16,000 boxes of dry materials.

The editors could encourage more descriptions and listings of museum collections and even rationalization and exchange of material. Small searchable databases on museum collections throughout the world might be coordinated through the newsletters. *Chrysomela* lists some members who are willing to identify particular groups.

What of the future? I know of no research newsletters in medicine and only one in biochemistry. Are these fields too competitive? Because research newsletters are cheap and foster cooperative research, the number will steadily increase providing that circulation is limited to genuine workers in the subject. Newsletters are especially valuable in the Third World and very small sums given to assist publication would be very effective aid. If or when we all communicate through computer networks, individual workers and those in the Third World will be left out in the cold: research newsletters could link computer networks with those outside.

Bibliographies, museum collections, stock collections, technical notes: research newsletters are a cheap and efficient way of promulgating information, even though they may be the untraceable publishing the uncitable. □

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New journals June 1985 to May 1986

CRITERIA for journals to be considered for review in this issue were circulated to publishers earlier this year, and were also published in *Nature*. They were that:

- the first number appeared, or the journal was re-titled, between June 1985 and May 1986 (although journals not covered in last year's review issue were also considered)*;
- the journal is published at least three times a year;
- the main language used is English;
- where possible at least four issues should be made available for review, including the first and the most recent numbers.

Last year's journals review supplement covered publications appearing between June 1984 and May 1985 and the second cut-off date, May 1986, allows for enough issues of a journal to have been published

*See *Nature* 323, 359–379 (1986). For previous journals review supplements see *Nature* 317, 293–308 (1985); 311, 309–330 (1984); 305, 477–497 (1983); 299, 491–514 (1982); and 293, 341–369 (1981).

for a reasonable sample to be available for judgement (many are quarterlies). A spread of four different issues is taken as the usual minimum on which reviewers' comments can be based.

It proved difficult to find reviewers for some, doubtless worthy journals, while other titles were considered to be of marginal interest to *Nature's* audience.

The brief given to reviewers was to limit themselves to comment on the publications sent to them, and to avoid discussion of general questions of periodical publishing. Opinions expressed in the reviews are based on a sample, and apply to mid-1987 at the latest. As in previous years, the preponderance of journals in the biological sciences reflects the bias of material submitted for review.

Details of editors and frequency of publication, and the subscription rates appearing at the top of each review, are given in most instances for 1988. This information is not complete in all cases, and readers interested in subscribing to a particular journal should check the rates with the publisher concerned. □

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		(Reidel).	

Bridging the electronic gap

Andrew Holmes-Siedle

Semiconductor Science and Technology. Editor R.A. Stradling. *Institute of Physics.* 12/yr. UK £160; elsewhere \$280.

Chemtronics. Editor J.O. Williams. *Butterworth.* 4/yr. UK £86; elsewhere \$98.

Journal of Molecular Electronics. Editors G.G. Roberts, R.W. Munn, G.B. Street, P.M. Chaikin, H. Bäessler and M. Aizawi. *Wiley.* 4/yr. UK £75; elsewhere \$145.

EUROPE is beginning to see itself as a unit, competing in the field of electronics against Japan and the United States. Because of the strategic importance of this activity and the wealth which it creates, it is as well that such cooperative activity is developing, albeit somewhat hesitantly. The three journals under review here all originate in Britain and have something to do with this new consciousness. The ESPRIT programme of the EEC is specifically picked out for mention by Professor Stradling in his editorial for *Semiconductor Science and Technology*. It is generally agreed that electronics, with its powerful scientific basis, is yielding a great deal of information. Some of this concerns the 'bits and pieces' which go to make up new electronic devices and is difficult to classify. Editors cannot always decide whether a paper falls under the rubric of electronic engineering, chemistry, metallurgy or physics — the reason, of course, being that modern electronics is a triumphant fusion of all of these fields.

These new journals attempt to bridge the old divide in which, for example, *Journal of Physics C* dealt with solid state physics and *D* dealt with applied. In the 1990s, even the interdisciplinary terminology of this decade will be outdated; some departments of materials science must be looking round for new names (has any considered Department of Chemtronics?); the present, select breed of 'device physicist' will be joined by 'device chemists' and 'device biologists'. The term 'molecular electronics' is indeed a pleasing and futuristic one: having found useful electronic activity in organic materials, we now have to understand how electrons move around within molecules as well as in the very large networks considered in classical solid-state physics. Suffice it to say that, in newly awakened Europe, new journals are needed to accommodate this ferment of ideas.

The editors of *Semiconductor Science and Technology* have to steer a line between the two existing Institute of Physics publications, *Journal of Physics C* and *Journal of Physics D*. Personally, I used to find those journals far less interesting than I now find this new one. This may confirm the new editor's view that, in accommodating papers on the *techniques* of solid-state electronics, the journal fills a gap. I would, however sound the warning

that — as for the Institute in general, so for this journal — the universities seem to predominate in its activities so far. For example, in the issues available for review, only 8 per cent of the papers were by authors in industry, although the editorial board is well balanced between the academic and industrial. There seems a good chance that the journal will ease the congestion in the area hitherto occupied by the *IEEE Transactions on Electron Devices*, *Journal of Applied Physics* and *Solid State Electronics*.

Chemtronics and *Journal of Molecular Electronics* can be compared fairly closely — both will be read more by chemists than by physicists, are more international than *Semiconductor Science and Technology*, and are perhaps trying to define a new interdisciplinary niche (*Chemtronics* describes itself as dealing with "topics at the interface of chemistry and electronics"). The scope of *Molecular Electronics* has already been mentioned. That of *Chemtronics* takes in a much wider range of chemical techniques. It seems to be a journal for those who really want to get their hands dirty at the chemical bench; by that I mean that preparative chemistry figures in the contents, especially chemical vapour deposition, an extremely important subject in the new electronics. There are practical articles on quality control and on the behaviour of the gases used in semiconductor processing. Scientists affiliated to chemical supply firms appear in the list of editorial advisors, although the fairly high price per page suggests that no subsidy is received from their employers.

By comparison, *Journal of Molecular Electronics* is a more limited affair, being half the price per page and having longer, more contemplative papers on organic compounds applied to devices or on the Langmuir-Blodgett film. The beauty of organic devices, if we can get them to work, is that we can tailor organic compounds with respect to electronic activity. The effect of adding a nitro- or amino-group to an organic molecule is at least as profound as the doping of an inorganic semiconductor, and in many other ways we have more freedom in the selection of device material. Of course, this also puts us directly on the road to macromolecular electronics, using proteins. Thus, this modest-seeming journal may hold more

keys to the future than does the more classically chemical one to which it is compared here.

Speed of publication is good in all three journals (short papers appear in about three months). Quality of production is also good; in particular, *Chemtronics* contains plentiful photographs, and the diagrams in *Journal of Molecular Electronics* are pleasingly bold. In the latter, I particularly welcome the editor's encouragement to contributors to "include enough introductory material to help readers . . . to appreciate the broad significance of the work reported". In other words, he is asking the authors to relax their accustomed space-saving style for a couple of paragraphs and let into the act a large number of readers who might otherwise be too puzzled to stay with the paper and understand it. In interdisciplinary journals such as this it is right that space limitations should be cautiously relaxed in the interest of better comprehension across fields.

All of these three journals will find a useful place in departmental libraries of physics, chemistry, metallurgy and biochemistry. Industrial researchers will also find in them the stimulation they need to produce the wealth-creating devices of the future. □

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Material progress

Lindsay Greer

Journal of Materials Research. Editor-in-chief W.L. Brown. *American Institute of Physics.* 6/yr. US \$260; elsewhere \$280.

IN THE United States, the meetings of the Materials Research Society cover a broad range of materials science and have encouraged a move away from traditional disciplines. *Journal of Materials Research*, an official publication of the society and following its aims, enters the field in competition with existing journals of materials science and with a number of metallurgical journals now increasing their scope.

In general, the quality of the articles is high. The aim of wide coverage has been met with contributions on the preparation, processing, properties, characterization and theory of materials. Ceramics and alloys have been well represented, but polymers and composite materials have not. A significant emphasis, setting this journal apart from its rivals, is on electronic materials, both semiconductors and contacts, and related processing. This emphasis is appropriate at a time when

much excellent research is being done in this area, and reflects that of the society's meetings and also the choice of editor-in-chief (C.B. Duke of Xerox for the first year, and now W.L. Brown of Bell Laboratories).

The study of microstructure is of particular importance in materials science, and the reproduction of micrographs (optical and electron) in the journal is of high quality. The printing and layout are clear, and the paper good. The listing of contents by topic and the materials index are worthwhile features. Refereeing takes an average of three months for full articles and two months for rapid communications, and appearance in print is only one or two months later. Potential contributors may also be attracted by the wide circulation to all members of the Materials Research Society, and to potentially greater numbers because the

journal is quite affordable for members of affiliated societies.

About 80 per cent of the articles come from the United States, and regrettably this proportion seems to be increasing. Authors from elsewhere may be deterred by the page charges (\$70 per page). It is hard to believe that these charges can make much of a contribution to costs; perhaps they are merely a device for limiting the length of papers, but that could surely be done more effectively and fairly by firm refereeing and editing.

That criticism apart, it is clear that *Journal of Materials Research* has already established itself as an important archival journal. It seems assured of a healthy future. □

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Considering clusters

Brian J. Howard

Zeitschrift für Physik D: Atoms, Molecules and Clusters. Managing editor I.V. Hertel. Springer-Verlag. 12/yr. DM 1592 plus carriage charge.

THE emergence of a new journal in atomic and molecular physics is usually difficult to justify. It is, however, debatable, whether or not this is a new journal because it arises from the division of the well-established and well-respected *Zeitschrift für Physik A: Atoms and Nuclei*. Atomic and nuclear physics make strange bedfellows and it has become customary in the physics literature, at least in the main general journals, to separate such diverse subjects (witness *Physical Review*, *Journal of Physics*, *Il Nuovo Cimento*, *Physica* and others).

A welcome aspect of the new journal is implied in its sub-title — *Atoms, Molecules and Clusters*. There are many journals devoted to atomic and molecular physics, but only a few editors appear to have fully appreciated the great growth of interest in clusters. This is a subject in which a wide range of questions, both physical and chemical, need to be addressed. Clusters are also of fundamental importance for the deeper understanding of the transition from isolated atoms and molecules, via large aggregates, to the properties of the condensed phases.

In his introduction, the editor of *Zeitschrift für Physik D* expresses the hope that "cluster scientists, in particular, will appreciate an obvious 'home' for this rapidly expanding field". Although I welcome these sentiments, they are certain to prove too optimistic. Other journals such as the *Journal of Chemical*

Physics already cater for the subject, and are where most major advances are still likely to be published.

The specified aim of the newcomer is to cover all aspects of atoms, molecules and clusters, including their production, spectroscopy, interactions and dynamics. However the early issues show a significant bias towards atomic physics, no doubt reflecting the journal's origins. There are, however, a few high-quality papers on molecules and clusters. There have also been special issues devoted to synchrotron radiation, metal clusters and multiphoton process, all of which have



helped to redress the balance. The editorial board has been carefully chosen to reflect all aspects of the proposed subject area; its members are distinguished scientists from a variety of fields and should attract a wider range of papers once the journal is more generally known.

The quality of production of the papers is high. There is a stated aim of reasonably rapid publication (three months) but the average appears to be closer to five months. There is also the opportunity to publish short notes produced from camera-ready typescript, which are guaranteed to appear in print within six weeks of receipt of the final manuscript.

There can be little doubt that this will become an established and presumably respected journal. It is perhaps not the journal one is most likely to browse through, but it will become a valuable source of information on the physics of atoms and molecules, and clusters. □

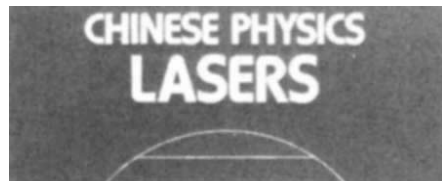
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In short, the news from China

I.N. Ross

Chinese Physics — Lasers. Editor Chinlon Lin. American Institute of Physics. 12/yr. US \$400, elsewhere \$410.

THIS journal is a translation of the *Chinese Journal of Lasers*. Each issue contains 10 to 15 main contributions, together with a number of shorter items called "Science Notes" and a number of "Letters".



Generally, the papers are short and report the results of novel experimental work in topical areas.

There are usually several (around four to five) papers with theoretical sections backed up with experimental verification, and one or two longer articles of an analytical and more substantial nature. The subject matter is strongly directed towards new developments in lasers and techniques used in lasers (56 per cent), but there are also contributions on nonlinear optics (14 per cent), optics and holography (20 per cent) and some reports on the (largely medical) applications of lasers.

The journal combines a strong experimental bias with a predominance of short articles, a lack of highly critical reviewing and an evident preference for papers which report initial and often useful results of new ideas. Few papers of this sort are likely to make important contributions to the subject, but a large number of readers, particularly those involved in all aspects of laser development and nonlinear optics, will find subject matter of considerable relevance to them. There are also sufficient contributions in optics and holography to form a worthwhile section of the journal. Applications could well have been better left out, perhaps to enable more rapid publication of the mainstream papers; it is a little surprising, given the brevity of most of the contributions, that the publication time — typically a year — is so long.

This journal can be recommended as containing good material from a country now making notable contributions to laser physics. It collects together new ideas in its fields and presents a practical assessment of them, and as such does fill a gap not satisfied by other publications. □

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Access to Armenia

H.M. Rosenberg

Soviet Journal of Contemporary Physics — Armenian Academy of Sciences. Soviet Editor G.M. Garibyan. Allerton, New York. 6/yr. North America \$430; elsewhere \$465.

This journal consists of fairly short (usually five or six pages) original papers, nearly all of them emanating from the various institutions in Erevan, the capital of the Armenian SSR. The scope is wide-ranging, covering theoretical and experimental work in many branches of physics.

In the five issues sent to me for review, the subjects covered include thin-film semiconductors, plasma physics, stimulated Raman scattering, hydrodynamics, X-ray diffraction, liquid crystals, non-linear optics, particle physics, semiconductor junctions, spin glasses and picosecond techniques. Many of the papers consist of calculations predicting the behaviour of rather specialized models, rather than discussions of general theories of physical phenomena. For light relief there is also the occasional eulogy on some eminent physicist who has broken through the octogenarian barrier.

The English translation is quite satisfactory. This is more than can be said for the general level of production, which even in a spirit of generosity cannot be

said to be barely more than adequate. The print is double-spaced typescript from the pre-word-processor era, with paste-ups of formulae and expressions from the original journal, and the paper is a matt yellowish duplicating grade that most schools would be ashamed to use to inform parents about a forthcoming sports day. The reproduction of the figures from the original journal is just about legible and the few half-tone plates that there are appear as variegated splodges of grey on grey.

In the English translation the pagination starts afresh with page 1 for each individual issue, so that when these are bound into volumes there will be endless confusion. In the Russian original the pages run consecutively through the issues that make up a volume in the conventional manner. Allerton Press — surely you can do better than this?

Speed of publication does not appear to be at a premium in the Armenian SSR. Most of the contributions seem to have taken at least a year to get into (Russian) print and there is about a further year's delay for the translation — the journal can hardly be said to keep you up to date with recent developments. That criticism apart, there are some interesting papers to be studied. So although this is not a journal for a small specialized library, it must surely find a place in the stacks of central repositories. □

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Living by numbers

William A. Light

Soviet Journal of Numerical Analysis and Mathematical Modelling. Editor-in-chief G.I. Marchuk. VNU. 6/yr. DM970, \$462.

As its title declares, this journal consists of English translations of selected Russian papers on numerical analysis and mathematical modelling. Numerical analysis is an active and diverse area in Soviet and Western mathematics, and no publication can provide truly comprehensive coverage of all topics or methods of approach to the subject. For example, there are some numerical analysts whose chief aim is the development of computer code for practical purposes, while others are much more interested in the mathematical foundations of the area and may know little about the associated computer codes.

The journal aims to publish papers which concentrate on the theoretical rather than the practical aspects. It is therefore inappropriate for someone who wants to find carefully described methods and/or computer code. Instead it will

appeal to researchers who are already mathematicians, particularly those with a strong interest in the theoretical aspects of numerical analysis; although from its title the journal might be expected to be divided roughly equally between numerical analysis and mathematical modelling, the copies that I looked at were biased towards numerical analysis.

Within this subject area, the early issues have a strong leaning towards differential equations, and towards partial differential equations in particular. Only six issues had appeared at the time of writing this review, so it is a little early to identify trends. It must also be said that the numerical solution of partial differential equations is a thriving area, and perhaps it is predictable that there is a preponderance of papers on this topic.

The quality of the journal is, in my estimation, very high. The editor-in-chief and the members of the editorial board are all of considerable standing in the Soviet academic world, and their early selection of papers is excellent. This is no surprise; choosing from a large number of Soviet authors in such an active field, one would expect to see, in the main, a good standard of research methodology and

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writing. There is, of course, a time lag between appearance of the papers in English and their original publication in Russian, but from a random survey this seems to be relatively brief — about two years on average. Most of the papers also appear to come from preprints of the Department of Numerical Mathematics of the Soviet Academy of Sciences.

These days, for many research establishments one of the important considerations in subscribing to a journal is that of price and value for money. This journal is comparatively expensive, even though the costs of translation must be taken into account. The six issues of Vol. 2 cost approximately £320 at today's rate of exchange (the price of the journal is fixed in deutschmarks). Each issue contains four or five papers of average length 12 to 15 pages. The standards of printing and translation are both high, and each issue is a pleasure to read.

This journal certainly fills a gap in the world of numerical analysis, and many researchers will now be able to become more familiar with important Soviet developments in the field. But the cost of that familiarity will be much higher than that of many Western journals in the same area. □

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Display of rivalry

Neville Boden

Liquid Crystals. Editors G.R. Luckhurst and E.T. Samulski. *Taylor & Francis.* 12/yr. UK £272; US \$490.

LIQUID crystals were discovered 100 years ago, but until the 1970s they remained little more than an academic curiosity. Today they are established as important materials and will become even more so in future. Clearly, there is a need for a specialist journal which focuses solely on this emerging discipline. The arrival of *Liquid Crystals* will, therefore, be welcomed by those working in the area.

The journal contains original research papers, preliminary communications and invited review articles concerned with all aspects of liquid crystal science and technology. This includes experimental and theoretical studies of liquid crystals, their synthesis and applications. The papers published to date are of the highest quality, as are the printing, illustrations (colour plates, too) and format; the style, in fact, is identical to that of *Molecular Physics* which is from the same stable. Papers may be published in English, French or German, but it would be wise for the editors to encourage the use of English whenever possible. The distinguished editorial board will be an encouragement to intending contributors, as will the aim to publish material rapidly: papers should appear approximately six months after their receipt and preliminary communications after four months. Good going!

Liquid Crystals will be in competition with *Molecular Crystals* and *Liquid Crystals* published by Gordon & Breach. At present there are many factors in favour of the newcomer. Printing-style and format are superior, and in my view it gives better value for money. The more established journal also publishes research papers on molecular crystals and low-dimensional solids, and these will no doubt be of interest to other users of the library. But all of those involved in liquid crystal research will welcome a new journal devoted to their own subject, and also the 50 free reprints of their published work.

The success of the journal will, however, depend upon whether it can establish an identity quite distinct from its competitor, whether it can attract the most original research papers currently being published in the primary journals of physics, chemistry and biology, and whether the publishers can maintain its comparatively reasonable cost. □

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Going along with the system

S.R. Wilbur

Computer Systems: Science and Engineering. General editor T.S. Dillon. *Butterworth.* 4/yr. UK £93; elsewhere \$101.

WITH the advent of low-cost processing elements, the nature of computer systems has changed radically in recent years. To improve processing performance or reliability, complex multiprocessor systems structures have been developed which are rapidly becoming the norm not only for industrial applications, but also for general-purpose and personal computing. Analysis and modelling of the hardware, the software structures and the architectures of such innovative systems are vitally needed.

The journal under review is targeted to meet this need. Its broad aim is to "publish high quality papers on theoretical developments in Computer Systems science and their applications in Computer Systems engineering". More specifically it expects to be an international journal covering at least: fault-tolerant computer systems, performance modelling, real-time computing, distributed computing, large software systems, specification of computer systems, system aspects of VLSI, computer networks and special-purpose system applications.

Judged by its first few issues, the dominant theme is performance analysis or modelling, with lesser coverage of fault-tolerant computer systems, distributed computing, real-time computing, testability, systems realization and computer

networks. The quality of papers is good, and the standard seems to have been maintained over the first 18 months. Yet to date most papers have been long (5–15 typeset pages) and the journal lacks the liveliness associated with short papers or a correspondence column. Although there is no evidence of the refereeing cycle time, the conference and book reviews published were somewhat belated; in such a fast-moving subject, delays of 9–15 months for conference reviews are unacceptable. The quality of production is high, with all the papers being typeset and the art work re-labelled. There is a stated intention of publishing tutorials and survey articles, but none were included in the issues I received.

In aims, *Computer Systems: Science and Engineering* is more quantitatively biased than the *ACM Transactions on Computer Systems*, and overlaps somewhat with *IEEE Transactions on Software Engineering*. The overlap with the IEEE journal is noticeable in the first few issues, but there do appear to be enough good papers for both journals at present. Nonetheless, it is a pity that *Computer Systems: Science and Engineering* has not strengthened its coverage of system realization and system aspects of VLSI design, which were parts of its declared scope and which are not covered by the IEEE journal.

Altogether this is a good-quality publication addressing the broad area of computer systems, with an emphasis upon performance modelling and analysis. But the conference reports should be published more quickly, and the inclusion of short papers and correspondence would help to give the journal a less sluggish air. □

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Across the fields

C.A. Brebbia

Transport in Porous Media. Editor Jacob Bear. *Reidel* 6/yr. Dfl. 375 (institutional); Dfl. 156, \$67 (individual).

THE stated objective of *Transport in Porous Media* is to provide "high quality refereed papers which describe research results that contribute to the understanding of and the ability to model transport phenomena for practical applications".

Although these phenomena occur in a multitude of disciplines such as civil, agricultural and chemical engineering, reservoir modelling, bioengineering and many others, there has been little communication between researchers in the different areas. This journal thus meets an important need by presenting a unified approach to all transport problems and

pointing out common features of the problems that arise in various fields.

The composition of the editorial board and the editor's own background contribute to give a mathematical emphasis to the journal, stressing innovations in both theory and applications. Papers published so far tend to relate to new developments in mathematical and numerical methods.

The whole region of transport problems in porous media is covered, including chemical as well as physical aspects. The variables under consideration are mass, momentum and energy in single- and multi-phase media. The emphasis, however, seems to be on geotechnical applications, and there has been a series of outstanding contributions on this subject by well-known researchers. The quality of presentation is excellent, making the journal pleasant to read.

In addition to standard papers the journal accepts letters to the editor and short communications, and regularly

TRANSPORT IN POROUS MEDIA

publishes book reviews and information on relevant conferences. It also encourages the submission of reviews on topics of interest to its readers, with the objective of presenting a critical survey of the work done so far and suggesting directions for further research.

Altogether, the journal can be counted a success. It provides an excellent forum for the interchange of ideas between those scientists and engineers who, although working in different disciplines, have a common interest in the phenomena associated with transport in porous media. □

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Use and abuse of the Earth

Brian Bell

Applied Geochemistry. Editor Brian Hitchin. Pergamon. 6/yr. UK and North America £65, \$105, elsewhere £86.44, \$137.84.

Applied Geochemistry was launched in 1986 as the official journal of the International Association of Geochemistry and Cosmochemistry. As its title suggests, it sets out to publish articles which illustrate how geochemical principles can be employed to solve problems related to exploration for Earth resources (fuels and minerals), pollution of the environment, agriculture, medicine and other related fields. The issues published to date contain contributions on an excellent spread of topics, and it is to be hoped that this will continue in the future.

Geochimica et Cosmochimica Acta, sister journal of *Applied Geochemistry*, is complementary in scope, dealing with more theoretical aspects of geochemistry and petrology. Authors should carefully assess the partition coefficient of their contribution with respect to the journal chosen, and ensure that their article is wholly compatible!

Applied geochemistry has developed considerably over the past few years and it is surprising that more journals have not surfaced in order to meet the demand for publication space. *Journal of Geochemical Exploration* dates back to 1972, and is a well-respected publication which deals successfully with exploration techniques involving geochemical studies for fuel and mineral resources. However, the remit of

Applied Geochemistry appears to be much broader, and it is this feature which should allow it to develop its own style and coverage. In particular, environment- and health-related geochemical investigations are taking a much more prominent place in the public eye, and should prove fertile fields over the next few years.

The journal consists of articles, both short and long, and the processing time appears to be as little as three months in some instances. Production quality is



uniformly high. Reviews, *per se*, do not appear to be part of the present remit; that is a pity, because I feel that the incorporation of this type of feature would open up the subject of applied geochemistry to a much wider readership.

A common criticism of journals, both new and old, is that of cost. Fortunately, through membership of the International Association of Geochemistry and Cosmochemistry (\$10 in 1987), individuals can obtain the journal for an additional \$15 per year. Otherwise, the journal is, in common with most scientific publications, expensive.

It is usual to hear complaints about the appearance of yet another journal. But I suspect that many Earth scientists, and in particular geochemists, will find in *Applied Geochemistry* a journal which deals with what interests them, and which will save them from having to thumb through a number of other publications. □

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Promise of riches from the east

Robert Shackleton

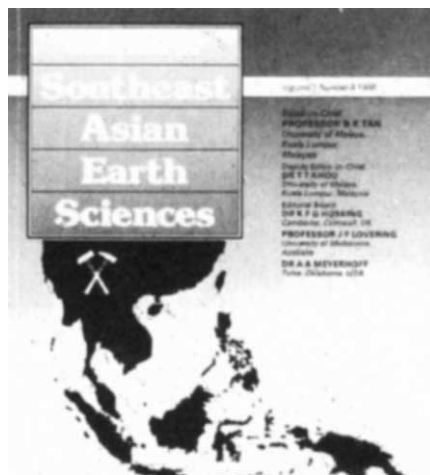
Journal of Southeast Asian Earth Sciences. Editor-in-chief B.K. Tan. Pergamon. 4/yr. UK and North America £45, \$75; elsewhere £61.02, \$97.30.

This new journal promises well. The region covered is of outstanding interest to Earth scientists, with its complex system of rapidly moving and rotating plates and microplates, and its immense mineral and hydrocarbon wealth. Literature on it has until now been scattered on a subject basis or buried in local publications in a variety of languages. The unifying view provided by plate tectonic theory implies that now, more than ever, it is important to see as a whole the evidence from many diverse branches of the Earth sciences. Regional as well as subject-

based journals are therefore essential.

Judging by the first four issues of Vol. 1 (1986), *Journal of Southeast Asian Earth Sciences* is well on the way to fulfilling that need. The balance and variety of topics is excellent: tectonics, oil and mineral resources, palaeomagnetism, seismic exploration techniques, petrogenesis, stratigraphy and palaeontology and more are covered in the first volume. The papers, usually about ten pages long, are well-written, well-illustrated and most are convincing. An exception is the long article, published as a special issue (Vol. 1, No. 3), on "The Palaeoposition of India". It is confused, inaccurate and out-of-date, and will do the reputation of the journal no good at all.

One purpose of a regional journal should be to enable those working in the



countries concerned to present their results to the world scientific community. Much of the basic data of geology comes from fieldwork and in the long run this will only be carried out by those who live there. The new journal has not yet tapped the resources of the indigenous scientists: one hopes that politics have not proved an obstacle. So far, most of the papers have come from scientists outside Asia. Without sinking into parochialism, a balance is needed between internal close-up views and external, often broad-brush interpretations illustrated with plate-tectonics cartoons.

Production, format and illustrations (even some, usefully in colour) are excellent. Dates of submission of papers are not given, but publication appears to be quick. Refereeing, however, needs attention.

The generally high quality of the papers and the exceptional interest of the region make this a journal that should be taken by all Earth science libraries, by organizations concerned with oil and mineral resources, and by many individual Earth scientists. A final attraction is that the price is reasonable. □

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Deposit accounts

Donald Hawkes

Ore Geology Reviews. Editor-in-chief K.H. Wolf. Elsevier. 4/yr. Dfl. 271.

This journal purports to be a sequel to the 14 volumes of the *Handbook of Strata-Bound and Stratiform Ore Deposits* issued during 1976, 1981 and 1985. Many of the review articles in those volumes encouraged further original research, and if this new journal attains the same standard it will be an essential addition to every major geological library. It is under the same editor, who has stimulating ideas about the nature and concept of review articles, so that the prospects of success must be rated as high.

Publication is intended as four issues per volume, and one volume per year, but a subscriber may not get four separate issues through the mail each year. In 1986, numbers 2-4 were included in the same cover; these constituted a special issue under a guest editor and consisted of updated papers originally presented at a symposium on "Metallogeny and Tectonic Development of Eastern Australia" held at the Seventh Convention of the

Geological Society of Australia in 1984.

The length of articles falls between that of the usual shorter journal reviews and comprehensive book-type coverage and, to this extent, the journal does fill a gap in the literature. The contributions are of high quality; some are definitive, but because they err on the long side and contain considerable detail they require a sustained commitment by a reader who has only a peripheral interest or is unfamiliar with the subject concerned. Unless the emphasis changes, this is unlikely to be a journal a reader will consult to obtain a rapid overview of developments outside his own field.

The print and page style is unremarkable Elsevier. Monochrome photographs, diagrams and tables are accommodated. Probably because the journal is new, admonishments to authors on the limitations set by the size and layout of the journal do not appear to be rigidly enforced. Large fold-out diagrams are accepted in exceptional circumstances.

Each article is accompanied by a passport photograph of the author(s), together with brief biographical details.

The title of the journal is unfortunate and will be misleading to many (?most) geologists because articles are solicited on almost any subject related to both metalliferous and non-metalliferous mineral deposits. Suggested topics include, for example, industrial minerals, pollution studies related to exploration and mining, "land based and oceanic studies", remote sensing, computer geology and exploration techniques. If the journal continues to publish thematic studies taking up two or more issues under a single cover, then a private subscriber might have to wait a considerable time before an article on his speciality appears. For the moment, I would suggest the best course is to borrow a copy from a library and hope that individual numbers may be offered for sale. □

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Sea changes of the past

Malcolm B. Hart

Paleoceanography. Editor James P. Kennett. American Geophysical Union, Washington, DC. 6/yr. \$100 (institutional), \$34 (individual AGU members).

THE subject area covered by *Paleoceanography* is a highly competitive one, which is already served by journals such as *Marine Geology*, *Marine Micropalaeontology* and *Marine and Petroleum Geology*. Initiating a new publication in this field would therefore appear to be a risky venture. However, *Paleoceanography* is currently attracting contributions of high quality. Its success no doubt stems both from the reputation of the editor and also from the low cost. The penalty for the latter is the use of camera-ready copy. Although many of the papers have reproduced well, others are less good, and in any case some readers are put off by a journal that looks like a typist's manual.

All of the issues sent to me for review contain papers of standard size and coverage. No reviews as such have been printed; nor have any short communications or discussions appeared. One issue (Vol.2, No.2) contained a thematic set of papers on Palaeozoic midcontinental seaways. One drawback of the camera-ready format is that there is no identifiable house style in terms of headings; for example, some authors use "Summary", others use "Conclusions". Perhaps this doesn't matter too much, but it does mean that reprints are not readily identifiable

as coming from the same source.

The illustrations can, however, be more of a problem, especially when using the double-column format. Authors (and this goes for other double-column journals as well) often fail to make diagrams fit either one column or two. The result is that either a lot of expensive paper is wasted or figures are overly compressed to fit into one column (see Droste and Shaver, Vol.2, No.2). In a subject area that relies heavily on maps and diagrams, editors must resist this temptation and give figures enough space. The other aspect that is a constant headache is that of half-tones, especially when they show palaeontological material. Photograph quality is very important and although the plates of Barrera *et al.* (Vol.2, No.1) are very good, those of Ciesielski and Grinstead (Vol.1, No.2) are really not acceptable.

Thus far the editor does appear to have a good flow of high-quality papers. I hope this will continue. But the competition for such papers is intense, and unless the photographic quality is consistently high, *Marine Micropalaeontology*, *Micropalaeontology*, *Journal of Foraminiferal Research* and other competitors will retain much of the palaeontological market. Although the loss of the biological input would not be fatal, it would indeed be unfortunate.

Ultimately, as libraries review their holdings and budgets, this journal may survive on price alone. But I would like to see it succeed on more scientific grounds. □

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Water works

John Lloyd

Journal of Contaminant Hydrology. Editors R.W. Gillham, G. Matthess, P.L. McCarty and P.S.C. Rao. Elsevier. 4/yr. Dfl. 286.

Journal of Contaminant Hydrology is a very welcome addition to the literature. It fills an essential gap by providing the opportunity to bring together scientific material in an increasingly important field; to date papers on this subject have been dispersed in a variety of less-scientific publications.

The title, however, is unfortunately somewhat misleading as the journal's scope emphasizes groundwater contamination, including the unsaturated and saturated zones but not surface water contamination unless related to groundwater. Investigations of the physical, chemical and biological processes that influence organic and inorganic contaminants will be considered for publication.

The contents of Vol 1 (1986–1987) are dominated by papers on organic contaminants, with Parts 1 and 2 devoted to a

special introductory issue on the topic. The volume includes a good mix of theoretical papers and applied case-studies, which one hopes will continue in future. The standard of the papers is high and readily comparable with the *Journal of Hydrology*, also published by Elsevier.

There appears to be no limitation on length of contributions, with most papers being some 10–20 pages inclusive of illustrations. The quality of production and illustration is of the usual high Elsevier standard, and the subscription price is reasonable. For the first volume the time elapsed between paper submission and acceptance was typically six to nine months. In addition to the scientific papers conference announcements are included.

Contaminants in groundwater pose some very real difficulties for scientists, engineers and administrators, and I hope that the editors will acknowledge the practicalities of the subject in future issues. I also hope that they will make the journal a truly international forum and get away from the North American bias of the first volume. □

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evaluate the details of the evidence.

Many of the papers, however, are disappointing — solid, competent pieces of research but with little spark or excitement. The authors have not asked themselves *why* they undertook this particular experiment, and they report their work in singularly depressing and turgid English. Developmental psychologists appear

COGNITIVE DEVELOPMENT

prone to imagine that a difference between performance at two ages is intrinsically fascinating. They are wrong. Yet some bright spots stand out, most notably a paper by Gelman and her colleagues on young children's numerical competence. One can actually hear the authors *thinking* about the interaction of principles and procedures that determines the acquisition of counting skills. For the rest, the journal has not yet achieved a style of its own, and the contributions would not be out of place in myriad other journals of child development.

Language and Cognitive Processes is edited from the Department of Experimental Psychology in Cambridge. It has a distinguished editorial board drawn from three continents, and it takes its 'cognitive' label seriously. Although the journal is devoted principally to the psychology of language perception and production, contributors are not afraid to draw upon linguistic theory for accounts of the structures that performance machinery encodes and decodes; neither do they hesitate to invoke the guts of sentence parsing by computer and the psychological validity thereof.

Most of the papers deal with core topics in lexical and syntactic processing by normal adults, and they include excellent work on languages other than English. Two fine studies focus on developmental issues: Miller on the conjunction of vocabulary learning and automated dictionaries, and Karmiloff-Smith on the changing linguistic forms that children deploy in cohesive narratives. The value of neuropsychological evidence for elucidating the functional architecture of cognitive skill is well illustrated by the investigation of Goodman and Caramazza into spelling impairment after closed-head injury; likewise, a case study of paraphasic errors enables Pate, Saffran and Martin to refine a model of normal language production.

Although some papers are tough going, the effort to follow them will be amply repaid. This journal has already secured a genuinely cognitive niche, hitherto vacant. □

John C. Marshall is in the Neuropsychology Unit, part of the Neuroscience Group at the Radcliffe Infirmary, Oxford OX2 6HE, UK.

Fruits of the tree of knowledge

John C. Marshall

Cognitive Development. Editor Wendell E. Jeffrey. Ablex, 355 Chestnut Street, Norwood, New Jersey 07648. 4/yr. \$58.50 (institutional), \$27.50 (individual).

Language and Cognitive Processes. Coordinating editor Lorraine K. Tyler. VNU. 4/yr. DM 197, \$94 (institutional); DM 134, \$64 (individual).

'COGNITION' has been the buzz word in psychology for the past 20 years, a notable span in a discipline where fashions wax and wane with the regularity of pop groups. Even now, no new title can avoid the incantation that spells a superior attitude to the study of mind. I sometimes think that before retiring to bed, modish authors and editors recite to themselves: "Lord, I thank thee I am not like other men, behaviourists, illogical positivists, or even like this stamp collector".

The original motive was that cognitive psychology should extricate itself from the follies of classical and operant conditioning and admit that a lot of very complex information-processing goes on inside the (even) average head. The guiding idea was that computationally explicit accounts of the mechanisms that underlie higher mental functions could be formulated and

LANGUAGE and COGNITIVE PROCESSES

rigorously tested. In the extreme case, people with a stake in such topics, albeit from different intellectual backgrounds, might even talk to each other once in a while, thus leading to a deeper integration of the more interesting aspects of experimental psychology, linguistics, philosophy of mind, logic, artificial intelligence and neuroscience. Outlets for such work are now many and varied. Hence the editor of any new publication must expect to be asked, "How is this journal different from all other journals?"

Cognitive Development is edited from the Department of Psychology at the University of California, Los Angeles, with an editorial board drawn mainly from well-known North American developmental psychologists. The journal reports experimental investigations of perception, memory, language, concept-formation, knowledge-acquisition and reasoning. Most of the studies concern the performance of young children; work on infants is not described in the first volume, although there is a sprinkling of papers on adolescents, young adults and the elderly. Book reviews and overviews of timely topics are included, and these are of a high standard; reviewers are allowed the space to weave an argument and critically

Systematic views

Adrian Friday

Cladistics: The International Journal of the Willi Hennig Society. Editors N.I. Platnick and C.J. Humphries. *Meckler, Grosvenor Gardens House, Grosvenor Gardens, London SW1W 0BS.* 4/yr. £80, \$95.

WHEN *Cladistics* first appeared in late 1985, its subtitle — *The International Journal of the Willi Hennig Society* — left no doubt in many minds as to what the content of the new journal was likely to be. There can be few biologists who are not aware of the controversies surrounding the development of cladistic methodology. Many of these controversies turn out to be less exciting than at first sight; rather, they are usually the result of misunderstandings and distortions.

A recent issue provides a statement of the nature of and need for *Cladistics*. The emphasis is on the scientific status of cladistic methodology (in the sense of "prediction, analysis and test"), its explicit nature ("... this new systematics does not have to rely on authority, tradition,

idiosyncrasy or irrational criteria") and its deployment in fields other than biology. The subject itself is summarized as dealing with "homology, its evaluation and parsimonious interpretation".

The journal aims to publish papers on theory, method, congruence studies and application studies in morphology, molecular biology, botany, zoology, linguistics, ontogeny, biogeography and systematic philosophy. Recent papers have ranged from the exclusively philosophical or mathematical to those dealing with detailed aspects of the comparative morphology and biogeography of particular groups. Not much molecular biology has so far been in evidence.

Since its origin the journal has been edited by N.I. Platnick of the American Museum of Natural History, a zoologist, and C.J. Humphries of the British Museum (Natural History), a botanist. The assistant editors are also one from each of these institutions. Each issue has approximately 100 pages made up of three sections: first the papers themselves, taking up about half or more of the issue, then a "forum" and finally book reviews. In the forum section some contributions are intended to provoke new discussion, others take up points made in papers

published in previous issues, and there are also reports of scientific meetings. No dates for receipt or revision of papers are given, but the turnaround seems to be relatively rapid.

One criticism of the journal is that it tends to be inward looking. Given its partisan nature it is perhaps not surprising that there are instances of homeliness, self-congratulation, condescension and sometimes descent into downright invective. These are the least welcome aspects of the journal's current style, but at least it

CLADISTICS

The International Journal
of the Willi Hennig Society

wears its heart on its sleeve and this does no great harm so long as it does not frighten away newcomers to the trade or those from non-biological disciplines whom the journal would like to attract. Papers critical of cladistics certainly have been published, but they are often followed, in subsequent issues, by forum contributions written in apoplectic incredulity — a sort of trans-Atlantic version of "Yours disgusted".

Cladistics, then, is nothing if not lively, and the editors must sometimes need to be very robust. In many ways the journal caters for cladists who previously would have relied on *Systematic Zoology* to publish articles and discussion of special interest to them. It has carried some substantial and fascinating contributions, and does clearly fill a publishing need. □

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Lines of sight

Stuart Sutherland

Spatial Vision. Editors-in-chief P.C. Dodwell and D.H. Foster. *VNU.* 4/yr. Price DM 250.

FOR many years scientific publishing houses have vied with one another to produce new learned journals, not without cause for it is a highly profitable enterprise. Now VNU Science Press has found yet another 'gap in the market'. *Spatial Vision* presumably means the processing of visual stimuli in so far as it is affected by their two- or three-dimensional arrangements in space, including the part of the visual field in which they lie.

As is still *de rigeur*, *Spatial Vision* is 'interdisciplinary', though it turns out that the different disciplines are separated only by a hair's breadth. They are visual psychophysics (the experimental study of the quantitative relationships between the physical stimulus and the experience it produces), cognitive or high-level visual processing and theoretical studies using computer simulation or mathematical techniques. Some examples of the three disciplines taken from the journal itself are, respectively, visual acuity, the recognition of simple figures, and computational methods of extracting the lines present in moiré patterns.

Perhaps the most laudable feature of

the enterprise is that the editors promise to make mathematical treatments available to the humble experimentalist. Unfortunately this is no easy task, and it is doubtful whether experimentalists will be fooled into thinking they have understood a piece of mathematics simply through the device of putting the more formidable equations in an appendix.

The articles appear to have been well edited and most are as clear as they could be given the difficulty of the subject. On the other hand the great majority stick to well-worn themes, such as the tilt after-effect or the number of spatial frequency channels. The results rarely come as a surprise. But in a notable exception, V.S. Ramachandran and V. Inada demonstrate that the motion seen in successive presentations of random dot patterns follows the motion of their edges if the edges are shifted or even of a background pattern of moving stripes. This is a further complication with which theories of motion detection will have to deal.

The journal is worthy and competent though expensive. But after all, who can blame publishers for making hay in the sunshine that remains before the arrival of the computer winter, when by tapping a few keys we shall be able to receive for nothing, or almost nothing, a VDU display of any paper we fancy. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex, Brighton BN1 9QG, UK.

At the bottom

Patrick D. Armitage

Journal of the North American Benthological Society. Managing editor Rosemary J. Mackay. *North American Benthological Society, 1,041 New Hampshire Street, Lawrence, Kansas 66044.* 4/yr. \$50 (institutional); \$15 (individual, NABS members).

THE North American Benthological Society (NABS) is one of the largest and most active groups of freshwater biologists in the world, and a journal affiliated to the society is long overdue. The journal's aim is to promote further understanding of benthic communities and their role in aquatic systems. Emphasis is placed on inland freshwater habitats but there is provision to accept papers on estuarine and marine topics. Theoretical discussions and critical appraisals of rapidly developing research fields are also considered, although none appeared in the first volume.

In the four issues provided for review a

wide variety of topics was covered in 30 papers. The largest single category concerned the life-history, growth and development of benthic invertebrate taxa. Others dealt with the effects of environmental disturbance on benthic communities, food and feeding of invertebrates, fish-invertebrate interactions, benthic algae, behavioural adaptations, physiology and taxonomy. The main emphasis of the journal appears to be on lotic habitats but I hope that later issues will attract papers on the lentic environment.

The first four volumes of the journal appeared as *Freshwater Invertebrate Biology*, which was acquired by NABS in 1985. The transfer of ownership was accompanied by the adoption of a broader editorial policy, and a change to a bright eye-catching cover and an improved editorial style with text, tables and figures clearly set out in two-column format. There are four issues a year, each containing six to eight good-quality papers averaging about ten pages in length. Each issue carries between four and seven book reviews. Publication time is rapid — most papers are in press within four to six months of submission. The subscription price is relatively low and the journal provides good value for money, particularly for individual subscribers (who must, however, be NABS members).

There are already several well-established journals publishing work on freshwater biology. Although none of them deals specifically with benthological problems, there will be an element of competition and the \$40 per page page-charge may discourage individuals and possibly institutions from submitting to the newcomer. However, the journal should continue to attract high-quality papers, particularly because of its association with NABS.

Most contributions originated from workers in the United States, and only two papers in the sample for review came from Canada. However, despite the 'North American' tag to the journal's title it is clear from the instructions to authors that papers from elsewhere are acceptable. I look forward to the appearance of such papers; perhaps they could address the similarities and differences between European and American benthos and benthological problems. A section for short communications, comment and reply to previous papers would be welcome to enliven the presentation.

In the editorial to the first issue in the new format the editor states that quality will not be sacrificed for quantity. A rigorous editorial policy has ensured that such is the case, and this journal will make an important contribution to the freshwater biological literature. □

Patrick D. Armitage is a Principal Scientific Officer at the Freshwater Biological Association's River Laboratory, East Stoke, Wareham, Dorset BH20 6BB, UK.

Biological issues in question

John R. Durant

Biology & Philosophy. Editor Michael Ruse. Reidel. 4/yr. Dfl. 81 (individual), Dfl 211 (institutional); elsewhere \$29.50 (individual).

As Michael Ruse observes in his first editorial, in the past the philosophy of science was practically synonymous with the philosophy of physics. Even as recently as the early 1970s, only a handful of philosophers (most notably Ruse himself and his associate editor David Hull) worked mainly or exclusively on the philosophy of biology. This was partly because philosophers tended to view physics as the paradigmatic natural science, and partly because they believed that physics raised more, and more interesting, philosophical problems than its sister disciplines.

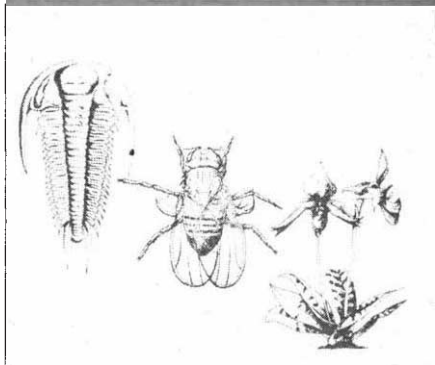
All this has changed over the past 15 years. With the spectacularly successful growth of biology, on the one hand, and the emergence of a number of major theoretical, moral and ideological controversies (particularly in the field of evolutionary biology), on the other, there has come the recognition that biology is every bit as scientific as physics, and that it raises just as many interesting philosophical issues. Today, there is a substantial and growing international community of what Ruse terms "thinking biologists" (what other sorts are there?), philosophers and others who share a concern to understand better the fundamental aims, methods, ideas and implications of biology.

Biology & Philosophy is intended to be the house journal for this scholarly community. Sensibly, the journal takes a generous view of its field and accepts not only mainstream philosophical but also relevant historical, sociological and other contributions. Its format is broadly conventional, with a mixture of topic articles, "target" articles plus multiple responses, book reviews and "booknotes". By the standards of much philosophy of science (which tends to verge on the obscurantist), its style is both accessible and engaging.

Some areas of biology currently attract much more philosophical interest than others. In Vol. 1, exactly two-thirds of the indexed contributions are concerned with evolution. This may seem excessive, but it is justified both by the central place of evolutionary theory in modern biology and by the wide range of important issues that are currently being debated in this area.

The first issue contains a helpful review article by John Collier on Daniel R. Brooks and E. O. Wiley's non-equilibrium

BIOLOGY & PHILOSOPHY



thermodynamic theory of evolution, a revealing *in vivo* analysis by Ullica Segerstrale of the sociobiology controversy, and a useful three-way discussion between the editors, George C. Williams and Elliott Sober, of Sober's big book *The Nature of Selection*. Later issues follow up several of these topics, and include discussions of the philosophical status of evolutionary theory, evolutionary epistemology, and that old and much-flogged war-horse, evolutionary ethics.

Despite the obvious emphasis on evolution, the journal covers many other topics. Early issues include articles on the biological species concept (are species classes or individuals?), ecological modelling, and teleology and reduction. In future issues, we are promised treatments of Plato on kinds of animals, the mind-body problem and the second law of thermodynamics, and the biological roots of morality.

Biology & Philosophy has started well. If it can sustain its initial seriousness and readability, and if it can avoid getting bogged down in the kind of ideological word-play that has bedevilled so much previous debate about, for example, "biological determinism" and "reductionism", it will perform a genuine service. For the time being, certainly, this is a journal that no "thoughtful biologist" should ignore. □

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Autumn books

The next review supplement to appear in *Nature* will be Autumn Books, on 19 November. Among the books scheduled for review are *Bones of Contention: Controversies in the Search for Human Origins* by Roger Lewin; *The Purpose of Forests* by Jack Westoby; *Quiddities: An Intermittently Philosophical Dictionary* by W.V. Quine; *The Oxford Companion to the Mind* edited by R.L. Gregory; and *Academic Freedom & Apartheid* by Peter Ucko.

Hi-tech down on the farm

Michael E. Moncaster

Computers and Electronics in Agriculture. Editor-in-chief J.R. Lambert. *Elsevier*. 4/yr. Dfl. 236.

OVER the past decade electronics and computing have become vitally important to agricultural research, development and production. Until recently, however, there was no journal dedicated to this area of application. *Computers and Electronics in Agriculture* aims to fill the gap.

Launched in October 1985, its policy is to cover any application of computers or electronics to instrumentation, control or management of agricultural processes, activities, equipment, enterprises or systems. Modelling, computer software and information processing are included where relevant to agricultural management, and also robotics for agricultural production. Agriculture is interpreted broadly to include forestry and veterinary medicine, as well as all aspects of crop and livestock production.

In the first four issues, published over a period of 17 months, papers covered a wide range of applications, even extending to the fishing industry. Robotics was one of the few subjects not touched upon. Although the journal is intended as an international forum, authors were from five countries only, and all but six of the 30 papers and notes were from North America. Europe was represented by British contributors only.

Editorial policy is to include original papers on scientific work, reviews of particular topics, technical notes on (for example) circuits, systems or programs, scientific discussions, letters commenting on published papers, guest editorials, book reviews and notices of international meetings. In practice, half of the papers are eight to ten pages long (3,000–4,000 words), and each issue contains on average about six papers, one of which extends to 17 or 18 pages. Five technical notes of five to eight pages have been included, as well as one review paper of 25 pages — on semiconductor device technology and digital system design — and two book reviews. As yet no letters, guest editorials, scientific discussions or notices of international meetings have appeared.

The scientific quality of the papers is high; no doubt refereeing by the appropriately qualified and diverse editorial board with representatives from ten countries helps. To what extent this slows down publication is not clear, but on average eight months have elapsed between acceptance and publication. The quality of production is similarly high; layout,

printing, illustrations, paper and binding are excellent. With about 500 words on a page 16 cm by 24 cm, legibility is no problem.

At a subscription of \$75 for the first four issues, this journal represents good value for a specialized publication. Its high quality in all respects and the success with which its aims are being met outweigh some shortcomings in the amount and type of information presented and in the publication delays evident in the first year or so. Because of its unique coverage of

a particularly important area, its chances of survival are good, provided that the quality can be maintained. Although the subscription has increased to \$100 for Vol. 2, it must remain on the essential reading list for all those involved in the application of computers and electronics to agriculture. □

Michael E. Moncaster is Head of the Information Engineering Division, Agricultural and Food Research Council Institute of Engineering Research, Silsoe, Bedfordshire MK45 4HS, UK.

Thrilled to the marrow

Elizabeth Jones

Bone Marrow Transplantation. Editors John Goldman and Robert Peter Gale. *Macmillan*, London. 6/yr. UK £75; elsewhere £100.

Bone Marrow Transplantation is a journal which provides a unique overview of both the experimental and the clinical aspects of allogeneic and autologous bone marrow transplantation (BMT). To say it has an international flavour would be an understatement; the editors and assistant editors are representatives of six countries, and the editorial review board of over 70 individuals is drawn from 18 countries. Understandably, the journal has already attracted papers of a high scientific standard.

The journal is published quarterly at present but will appear bimonthly in 1988. Each volume usually contains an editorial, review articles, case reports, a large



number of first-class papers (which are usually published within six months of submission) and announcements of future meetings. So far, however, there has been only a regrettably limited amount of correspondence.

The review articles cover a wide variety of topics such as the use of circulating stem cell allografts in BMT, the use of cyclosporin to prevent graft-versus-host disease (GvHD), analysis of BMT in man during the past 18 years, and the role of BMT in thalassaemia and in multiple myeloma. The problems of using unrelated donors in BMT are covered and future trends in gene therapy are discussed. With gene therapy there is the exciting prospect of inserting normal genes into bone marrow stem cells before autologous BMT of corrected marrow for the treatment of

haematological malignancies (leukaemia), enzyme deficiencies (severe combined immunodeficiency disease) and haemoglobinopathies (sickle cell disease, thalassaemia).

The donor of choice for an allogeneic BMT is an HLA-identical sibling, or a one-haplotype-matched family member, but HLA-matched unrelated donors are being increasingly used. The first editorial in Vol. 1 enumerates four areas in which BMT survival must be improved and most of the papers cover these areas. First, donor selection must be improved, particularly in transplants using HLA-mismatched family donors and unrelated HLA-identical donors, by using more sensitive matching of donor and recipient. The importance of matching for Class III antigens (C2, C4, Bf), for minor histocompatibility antigens, and the use of cDNA probes direct against the HLA genes are mentioned. Given the importance of the HLA system in BMT, there is a notable lack of papers in this area in the journal.

Secondly, improved conditioning regimes are needed and the relevance of TBI doses, busulphan and cyclophosphamide are discussed in the journal. Third, GvHD occurs in 45 per cent of HLA-matched recipients and in 75 per cent of one-haplotype-matched recipients. Techniques to reduce GvHD are discussed, such as *in vitro* treatment of marrow with soy bean agglutinin, monoclonal antibodies (Campath-1, IgM), albumen gradient sedimentation, E rosetting and treatment of patients with cyclosporin, methotrexate, and psoralen with ultraviolet light. Lastly, treatment of infection in BMT patients with toxoplasmosis, herpes viruses, cytomegalovirus and varicella zoster is adequately covered.

The articles and reviews are presented in a clear, concise fashion. This, together with the high-quality figures and illustrations, makes *Bone Marrow Transplantation* understandable both to specialists in the field and to the less expert. The latter in particular should be encouraged to read this journal. □

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Moving problems

J.P. Paul

Clinical Biomechanics. Editors A. Kim Burton and Jon H. Thomson. *John Wright*. 4/yr. UK £46; North America £99; elsewhere £53.

THE stated aim of *Clinical Biomechanics* is "to encourage interdisciplinary communication of the clinical aspects of biomechanics related to dysfunction of the musculoskeletal system".

Of the papers published in its first year, approximately 50 per cent deal with the lumbar spine, its morphology, its pathologies, its functions in lifting and other ergonomic aspects. Approximately 20 per cent of the remaining articles relate to the loading of joints in the limbs in normal and pathological situations. Other papers deal with gait, endoprostheses, pain, fracture and other injuries and rehabilitation. Selections of abstracts from other journals are also published; *Spine* is easily the most cited, followed at a long distance by *Ergonomics* and also *Scandinavian Journal of Rehabilitation Medicine*. It remains to be seen whether the journal maintains this bias towards problems of the lumbar spine, or moves to more general coverage of material in accordance with its aims.

The papers are medium to short in length, without extensive mathematical analysis, and concentrate on biomechanical measurement and clinical assessment. Each issue has an editorial comment on a relevant topic, along with book reviews.

This is a high-quality production, with clear type and good illustrations. Generally the photographs are in black-and-white, though sometimes colour is used. I felt, however, that the line diagrams and graphs were too often straight copies of computer-screen images or computer print-outs with corresponding deficiencies of the lettering. Also, in the captions there is some inadequacy in descriptions of quantities presented.

The material published has included unrefereed papers from symposia and letters to the editor about previous articles. The papers themselves appear to reach printed form between three and seven months from first submission.

To my mind, the scope of *Clinical Biomechanics* lies between that of *Journal of Biomechanics* and that of *Journal of Bone and Joint Surgery*, with a leaning towards the latter. The papers appear to be carefully reviewed and proof-read. Advertising is included, and the price is reasonable: the cost per page, averaged over the year, is in the middle range for comparable publications. □

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Work in disorder

Alan N. Davison

Metabolic Brain Disease. Editor-in-chief David W. McCandless. *Plenum*. 4/yr. US \$47.50 (individual), \$115 (institutional); elsewhere \$55 (individual), \$129 (institutional).

THIS new journal is intended for publication of original high-quality contributions on both clinical and basic aspects of disorders involving brain metabolism. Each issue contains a review of up to 26 pages (about 600 words per page) and some six original papers of varying length. Short book reviews and occasional correspondence will also be published. There are no page charges, and it is intended that publication will be rapid (eight months from the time of acceptance). The journal is of a small, attractive format, and the layout of

the contributions is excellent.

The editorial board is predominantly American, with the chief editor's office in Houston, Texas. In the first volume most of the articles originate from the board itself and, predictably, are of a good standard. They cover a wide area, ranging from inherited neurological conditions to neurotoxicity in animals. Nevertheless, all the papers could well have been published in existing publications such as the *Journal of Neurochemistry*.

With time, perhaps, the scope of the journal will be more focused, for example on aspects of energy metabolism and its relationship to neurological disease. I would also suggest that the reviews need to be carefully selected and critically edited with the specialist rather than the general reader in mind. □

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Liverish exchange

A.E. Read

Journal of Hepatology. Editor-in-chief Dame Sheila Sherlock. *Elsevier*. 24/yr. Dfl. 740 plus carriage charges.

UNTIL 1985 there was only one specialist journal of hepatology in the English language. So the arrival of a second — the *Journal of Hepatology*, Journal of the European Association for the Study of the Liver (EASL) — was timely.

The editor-in-chief is Dame Sheila Sherlock, a distinguished clinical and experimental hepatologist. She is supported by an editorial board of 40 colleagues, who represent hepatology worldwide though most of them come from Europe. The standard of presentation is good, and the photographs — occasionally in colour — and other illustrations are clear.

The contents of the journal consist of original scientific papers, reviews, book reviews and announcements of interest to hepatologists (meetings and so on). There are usually 12–15 papers in each issue; topics are widespread and range from basic science to clinical practice, with the accent on the latter. The most popular subjects are portal hypertension and virus B hepatitis. This is likely to reflect the fact that these topics are probably the main growing points in hepatology generally, rather than indicating any pre-selection. It is noteworthy that the period between acceptance and publication of papers is consistently less than six months which is a major achievement. The papers are of rather variable quality but, overall, in view of the large number of referees (nearly 260 are listed as looking at papers which

appear in Vols 2 and 3), such an international refereeing system is praiseworthy though probably cumbersome.

Book reviews are usually confined to one or two volumes per issue and are fairly brief (one wonders, incidentally, how much would-be purchasers are deflected by a bad review, which in any case usually appears long after the would-be purchaser has made up his own mind). Most issues also contain a review-type article, though these are usually not particularly long and certainly not over-endowed with references. There is however a place for the briefer review article in a journal whose main function at the moment is to offer a place for clinically based scientific papers.

What does *Journal of Hepatology* not



offer? There is no editorial comment on the papers that are accepted — which is reasonable — but neither is there a section for comments by readers on papers that have been published. This is a deficiency that needs to be corrected.

Though youthful, the journal does seem to be filling a need in that it is attracting and publishing quickly a variety of investigative and clinical papers on hepatology. It perhaps needs to find a particular direction — such as viral hepatitis — if it is to attract quality papers away from other general and gastroenterology journals. It should also allow for comments from outside about papers to be published, and it needs to be cheaper if it is to appeal to medical librarians. Presumably, though, EASL is its captive audience? □

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Colourful grounds for separation

George H. Elder

Biomedical Chromatography. Editors-in-chief Elizabeth F. Hounsell and C.K. Lim. Heyden, Spectrum House, Hillview Gardens, London NW4 2JQ, UK. 6/yr. UK £120; elsewhere \$180.

THE title of this new journal reflects its contents. Each issue contains up to a dozen concise papers describing chromatographic methods for the separation of substances of biological interest and commenting on their applications; reviews are also published occasionally.

Many of the papers have a clinical slant and there is a strong bias towards high-performance liquid chromatography. But the range of analytes discussed is commendably wide; the editors have included papers on proteins, peptides, amino acids, carbohydrates, steroids, porphyrins and vitamins, while managing to control the advancing tide of drug separations sufficiently to preserve balance.

The production quality is high, the turnaround time of six months or under is good, and the editors appear to have achieved their aims of attracting contributions from many countries and encouraging proper description of methods. I could find none of the all-too-frequent accounts of HPLC separations in which the description of solvents is so vague as to almost leave to the reader the task of devising the method.

Chromatographic separations are central to much biomedical research and to many diagnostic and monitoring methods. Where does one turn when faced with a separation problem? Most of us hope that it will have already been at least partially solved and consult colleagues and the literature. The latter contains journals devoted exclusively to chromatography, analytical journals with a wider scope and the methods sections of papers in general journals.

Apart from extending the opportunities for publication, does *Biomedical Chromatography* have anything to offer? Most of the papers, as is perhaps to be expected from the way that method-orientated research now operates, are in the "state of the art" rather than the "never separated before" category. There is a need for a journal emphasizing medical applications of chromatography; but the competition is fierce from established, though less specific, publications. High standards and prestige will be required: *Biomedical Chromatography* shows promise. □

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Image promotion

George K. Radda

American Journal of Physiologic Imaging. Editor-in-chief Maynard L. Freeman. Alan R. Liss. 4/yr. USA \$95; elsewhere \$108.

THIS new journal is aimed at "an audience interested in the study of physiology and pathophysiology by imaging techniques and their effects on the practice of medicine". The editorial in the first issue also states that the journal intends to cut across traditional disciplines.

Both of these aims are highly laudable, and if achieved will certainly result in a scientific periodical that differs from the highly specialized journals publishing papers on different aspects of medical imaging. The emphasis on physiological investigations is also welcome. The brief editorial statement in the fourth issue, reporting a discussion by the editorial board as to whether the term "physiologic" was too restrictive and whether

biochemical, metabolic etc. should be added to the title, indicates the concern of the board to include papers on functional investigations in the broadest sense.

The papers published are original articles and "Current Concepts — Review Articles" (two reviews per issue). The journal is well printed, and with the high-quality production had a good start. Some papers have been accepted rapidly (within a month of submission), and overall publication times are very commendable.

To date, however, the spread of the papers has not been as broad as the stated aims. Most contributions are in the general field of nuclear medicine with some limited contributions in nuclear magnetic resonance, computed tomography and echocardiography. It would be sad if the editorial board cannot in future attract papers outside the use of isotopes. On the whole, the journal in its present format is good value even if I intensely dislike the term "physiologic imaging". □

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Fertile imagination

C. R. Austin

Human Reproduction. Executive editor R. G. Edwards. IRL. 8/yr. \$160 (institutional); \$80 (individual).

HUMAN procreation has become a fascinatingly complex research area, ranging through a broad spectrum of medical and biological interests. Problems being studied include sexual differentiation and development, reproductive and social behaviour, fertility limitation and promotion, gamete and embryo manipulation and storage, pregnancy induction and maintenance, fetal physiology, birth processes and diagnosis, and the prevention and treatment of birth defects. And most of these topics also have their comparative, legal and sociological aspects.

Human Reproduction is designed to provide a forum for the exchange of information and ideas on all these matters. Acceptable material takes the form of reports of original basic and clinical research, reviews of books and other technical publications, verbatim reports of select assemblies (for example those of WHO on "Artificial Reproduction", and of the Council of Europe on "New Techniques in Human Fertilization and Embryology" and "The Use of Human Embryos and Fetuses"), and commentaries and short communications. In addition, this journal is the official organ of the European Society of Human Reproduction and Embryology, and

special supplements present the abstracts from the annual conferences of the society, and occasionally the conferences of other relevant groups. Publication is only in English, but with the commendable editorial policy that poor English is not in itself grounds for rejecting a paper, and that every effort is made to render the effusion comprehensible.

Human Reproduction was born in January 1986. It is edited by R.G. Edwards, the prime mover in successful human *in vitro* fertilization and embryo transfer, aided by associate editors and an editorial board of Western Europeans known for

Human REPRODUCTION

their contributions in the journal's area of interest. It is a good-looking product, roughly A4 in page size, with two columns of clear print that makes for comfortable reading, and high quality half-tones and line drawings. Each paper begins with a helpful summary in bold type.

Articles I selected for close inspection all seemed well worthy of their inclusion. I was also favourably impressed with the speed of publication — the 131 refereed communications in the first 11 issues appeared in print on average 3.7 months after receipt. In brief, my opinion is that the circulation of *Human Reproduction* deserves to grow in a manner consistent with the journal's title. □

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Pharmaceutical progress

S.S. Davis

Anti-Cancer Drug Design. Editors Stanley Crooke, Stephen Neidle and Shigeru Tsukagoshi. *Macmillan, London.* 4/yr. UK £55; elsewhere £75.

Drug Design and Delivery. Editor J.S. Morley. *Harwood.* 4 issues per volume. \$286 (institutional); \$194 (libraries).

THE greater understanding of the biology of cancer at the molecular level has meant that it is possible to take a more rational approach to the design of anti-cancer agents. Such an approach employs the tools of modern molecular and structural biology as well as the more traditional methods of pharmacology, medicinal chemistry and biophysics.

Anti-Cancer Drug Design has been introduced to provide a unifying forum for these developments. The articles in the issues available for review are wide ranging in scope. Some of them present details of conventional medicinal chemistry, with descriptions of the synthesis of new analogues and their *in vitro* and *in vivo* assessment, while others consider aspects related to the effective delivery of anti-cancer agents to include antibody-targeted drug-carrier conjugates and drug-loaded microparticulates.

So far the journal appears to be achieving its objective of publishing research papers that involve the application of a rationality of approach rather than the mere listing of compounds synthesized and tested. Clinically orientated material, although welcomed, has yet to make a strong showing. The papers are in the form of conventional articles as well as short communications, and are generally of high quality. One 'mini' review has appeared so far; this was most informative and I hope that further such reviews will appear in forthcoming issues. The journal is well produced and so far the papers have been published rapidly after acceptance.

As with any new venture, it is vital that the initial enthusiasm generated by the editors does not wane and that potential authors do not revert to their previous outlets for publication. The field of cancer research is well served by a variety of journals and the same is true of medicinal chemistry; indeed, another journal that cuts across some of the material has also been introduced recently (*Cancer Drug Delivery*, published by Mary Ann Liebert Inc.). Today, the survival of any new journal is doubtful unless it fills an obvious gap. *Anti-Cancer Drug Design*, published with support from the Cancer Research Campaign, has a better chance than most.

In justifying *Drug Design and Delivery*,

the other journal reviewed here, the editor claims that he is "fulfilling a need, not catered for in the existing scientific press". Once again, the rationale is to provide a multi-disciplinary setting for concepts arising from chemistry, biochemistry, pharmacology, immunology and even mathematics. In doing so, it is hoped to create a comprehensive source of current information on innovative approaches to drug design, absorption, distribution, metabolism, release and finally clinical effect.

Whether such a gap actually exists is debatable. *Journal of Pharmaceutical Sciences* and *Pharmaceutical Research* could be said to have exactly the same intentions. Interestingly, it is hoped that the editorial policy will attract papers from individual scientists in the pharmaceutical industry who have been reluctant to publish in the past. It is certainly true that the publication policies of many pharmaceutical companies are over-cautious, but how the present journal would alter the situation is not explained.

The papers that have appeared to date



have been of mixed quality, and have dealt either with drug design or with delivery. Few have yet combined the two. The standard of presentation is generally high, and it is good to note that colour, when an integral part of the research, is published without charge to the authors. Papers are either typeset or, for rapid publication, are in a camera-ready form.

The one book review that has appeared so far (in the first issue of the journal) considered the book entitled *Multi-dimensional Pharmacochimistry* by P.P. Mager. The reviewers were not overly impressed by what they read. Most unusually, the author takes the reviewers to task in a letter to the editor in a subsequent issue. These two contributions make far more interesting reading than many of the scientific contributions.

Only time will tell whether this journal is able to establish a place for itself within the pharmaceutical sciences. Better quality papers need to be attracted, and the editorial board could play a role here. Three of its members are scientists who have had prestigious careers in the pharmaceutical industry and have now moved into academia; perhaps they could encourage their former colleagues to publish more! One member of the board has certainly been of considerable benefit — of the 35 papers published so far, he is the co-author of ten. □

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Being industrious

Colin Webb

Bioprocess Engineering. Managing editor H. Brauer. *Springer-Verlag.* 4/yr. DM 196 plus carriage charge.

THERE are currently more than 250 periodicals world-wide which serve the broad field of biotechnology, with new titles emerging by the month. Remarkably, for a technology with such commercial potential, very few of these deal with process engineering. Indeed, apart from *The Biochemical Engineering Journal*, which is published jointly with *The Chemical Engineering Journal*, and *Advances in Biochemical Engineering*, which is published annually, there are no English-language periodicals dedicated entirely to such matters. *Bioprocess Engineering* therefore fills an important gap in the literature. It publishes original papers, short communications and occasional review articles, on "all technical and economical aspects of processes in which natural or derived biological substances are the basic material".

In line with most quality journals, *Bioprocess Engineering* is produced in a two-column, A4 format, with all the text being typeset and figures being reproduced directly from the authors' originals. Proof reading of articles is the responsibility of the individual authors, which is just as well because the journal's own proof readers are guilty of fairly serious errors, from time to time, in the contents pages and index lists. Happily, pre-publication delays are not apparent, and most contributions appear within six to nine months of receipt of the manuscript.

The technical content is generally of a high standard, with most contributions keeping within the intended scope of the journal. Although, in its first year, 30 per cent of the papers came from the editors or their advisory board, none appeared to be merely page fillers and all presented useful new design information. As any industrial technology comes of age there becomes an increasing need for sound engineering evaluations of available information. Articles such as that by Schumpe and Deckwer (Vol. 2, pp. 79–94) fulfil such a need by not only supplying valuable new information but also by critically reviewing, and correlating, existing information from the literature. Such contributions, along with the many others of similar quality, make *Bioprocess Engineering* an invaluable addition to any biotechnology or process engineering library. □

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On the small side East and West

Stephen Oliver

Journal of Industrial Microbiology. Editor-in-chief G.E. Pierce. Elsevier. 6/yr. Dfl. 435 plus carriage charge.

Molecular Genetics, Microbiology and Virology. Soviet editor P.N. Burgasov. Allerton, New York. 12/yr. \$425 (North America), \$470 (elsewhere).

Soviet Biotechnology. Soviet editor V.A. Bykov. Allerton, New York. 6/yr. \$385 (North America), \$420 (elsewhere).

THESE three journals — one, *Journal of Industrial Microbiology* (JIM) published for the US-based Society for Industrial Microbiology, and the other two cover-to-cover translations of Soviet journals — present the reader with some obvious contrasts. JIM is produced to the usual glossy Elsevier standards and has a panel of editors drawn almost exclusively from the United States, many of them from well-known companies. The Russian translations are produced as camera-ready typescript; although this in itself is quite acceptable, the half-tone photographs have been photocopied from the original journal and are of such poor quality as to be totally indecipherable. The standard of the English used is generally good, but the prose can be turgid at times and it is impossible to say whether this is the fault of the translator or the original authors.

JIM covers an area of science which, although currently very active, is well served by existing journals (some of them, for example *Applied Microbiology* and *Biotechnology* and *FEMS Microbiology Letters* being the products of the same publishing house). Most of the papers are fairly short and some barely qualify as microbiology, for instance that dealing with the effects of aflatoxins on mice.

The subject matter of the papers covers a very broad range, but there are some dominant themes. Contributions on environmental microbiology and waste treatment make up the largest single class and may reflect the fact that this area is less fiercely competitive than some others in the commercial arena. On the other hand, biochemical conversions of antibiotics is another popular topic and can hardly be said to lack immediate commercial relevance. All the work presented relates to the bacteria and fungi, with no papers at all on viruses, algae or protozoa. JIM appears bimonthly, and although publication times began at about two months from final acceptance they soon fell to four months; the journal is thus unlikely to gain a place as a vehicle for rapid publication.

Altogether, I feel that JIM will have to

develop some distinctive role if it is going to survive against the competition. The intention is to publish short reviews, but the one example in the issues I read (on heterologous gene expression in *E. coli*) was disappointing. The editors will need to commission such reviews from acknowledged experts, rather than rely on voluntary submission, if an adequate standard is to be maintained.

The journal's real role, I think, will be to encourage microbiologists working in industry to publish their work. Attitudes to publication within companies have undergone a great change since the arrival of the genetic engineers, and it would be good to see industrial scientists unlock some of their collective wisdom in the areas of microbial biochemistry and physiology.

An obvious problem with the other two journals reviewed here is that all the papers come from the Soviet Union. It is simply not possible for any single country to produce a journal of international standard from its own resources, given the choice of publications open to authors these days — even the US-based JIM draws 30 per cent of its papers from foreign sources. The parochialism of *Molecular Genetics, Microbiology and Virology* (MGMV) and *Soviet Biotechnology* (SB) even extends to the bibliographies of the papers themselves, with a number of authors quoting only Soviet material.

In the issues I read, bacterial genetics was the dominant subject to MGMV, together with virology as related to

vaccine production. There were a number of useful short reviews, such as one on microcins and another on bacterial mutagenesis (the latter being the sole contribution from a Western author). But on the whole, MGMV contains little to excite.

I found SB far more interesting. The journal covers the whole range of biotechnology, from molecular genetics to chemical engineering, and the papers are short (3–5 pages). The obvious analogue among existing journals is *Biotechnology Letters*, but although SB has something of that publication's liveliness some of the papers present work which is not novel and which would not have been accepted in a Western journal.

Of great interest to watchers of Soviet science are the lengthy reports of speeches by ministers, telling how biotechnology fits into the next five-year plan. We learn, for instance, that the single-cell protein programme is forging ahead but that one hapless institute has failed to produce a single new drug in 12 years, in spite of the expenditure of some 50 million roubles. We also learn that biotechnology will produce "profound qualitative changes in the substance and character of labour and in all the material and spiritual conditions of life". Biotechnologists in the West will hope that such sentiments are also close to the hearts of their own political bosses. □

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Place for overall immunity

Fred S. Rosen

International Reviews of Immunology. Editors Heinz Kohler and Constantin Bona. Harwood. 6 issues per volume (see below). £52, \$64.

IMMUNOLOGY is an unabatedly vigorous field of research, and some of the most important findings are published in non-speciality journals — *Nature*, *Science*, *Cell*, *Proceedings of the National Academy of Sciences* and so on. This leaves a need for publications which pull together the flood of new facts and findings. Most review journals have an unfortunate lag

Harwood Academic Publishers and Gordon and Breach Science Publishers produce journals under the 'flow system'. Once sufficient papers are accepted to complete an issue, that issue is closed and quickly published. According to the publishers, this avoids the usual delays created by rigid schedules, but means that the number of issues per year cannot be accurately predicted.

period from submission to publication. Perhaps this problem can in part be solved by the appearance of *International Reviews of Immunology*.

The journal is edited by Heinz Kohler and Constantin Bona, and a personal subscription represents good value. Each issue is devoted to a subject of current interest such as idiotype vaccines, T-cell clones, isotype regulation and so forth. Some issues are assembled by authoritative guest editors. A prestigious international editorial board has been recruited for this effort and its members should assure the future appearance of broad and comprehensive surveys of various immunological topics.

So far the scientific quality of the contents has been good. The format is of a convenient size, and the figures and illustrations (including some in colour) are well done. Altogether this publication is a useful addition to the small cohort of immunology review journals and annual reviews. □

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Pretty proteins

C.D. Rodger

Proteins: Structure, Function, and Genetics. Editor-in-chief C. Levinthal. Alan R. Liss. 8/yr. US \$150; elsewhere \$176.

AT FIRST sight, the very title of this new journal may seem surprising; overwhelmed by the advances in work on nucleic acids, one could be forgiven for thinking that protein research is no longer paramount and can be catered for by the existing journals of biochemistry and molecular biology. However studies of proteins continue to produce exciting information: three-dimensional structure determinations have been speeded up by the use of synchrotron radiation for X-ray diffraction, our ability to manipulate nucleic acids has enabled easier determinations of amino acid sequences, and site-directed mutagenesis provides methods of manufacturing synthetic polypeptides. There is no shortage of material for a new publication in this area.

Proteins first appeared in September 1986, and was intended to appear monthly. This schedule slipped, however,

PROTEINS

Structure, Function, and Genetics

apparently because of a lack of high-quality contributions. Each issue contains approximately ten papers, sometimes including an initial review article. The format of the contributions follows a good scientific style and includes a useful abstract. The journal appears to cater mainly for full papers and initially no short contributions of the "letter to the editor" type were included, though there has been some editorial comment. Shorter contributions are invited for a "comment" column but this has not yet materialized.

I found it difficult to classify the papers into the subcategories of structure, function and genetics, but a rough analysis of the four issues sent to me for review indicated that the journal was mainly attracting papers dealing with protein structure. This does not mean that this is a crystallographers' journal, however, for there are few of the standard "structure determinations at 2.5 Angstroms resolution" articles to be found elsewhere. There was reference to protein genetics in about a quarter of the contributions, either from the evolutionary standpoint or in terms of mutagenic approaches to produce "designer proteins". Further papers in the latter area especially could help the journal carve out a useful niche for itself.

The standard of production is excellent. The journal is attractively bound in a

coloured cover and is printed on glossy paper. The text is large and easy to read, as are the graphs and tables. The paper quality allows for good reproduction of photographic material and colour has been used to very good effect, especially in depicting computer simulations of three-dimensional protein structures.

The editorial board is composed of some 40 distinguished figures from the general area of protein research. Although most are based in the United States, the board does include representatives from eight other countries and approximately one-third of the contributions to the four issues were from

institutions outside the United States.

My overall impression of the papers published so far is that they are of high academic quality. The time between submission and publication seems quite short at present, and this should stimulate the flow of contributions. The breadth of coverage means that the journal should appeal to a reasonably wide audience, and if the quality of the articles is maintained, and the publication schedule adhered to, *Proteins* should do well. □

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Budding with promise

Peter A. Meacock

Yeast. Editor S. G. Oliver Wiley. 4/yr. UK £60, elsewhere £110.

NOWADAYS no self-respecting biologist or biotechnologist who professes an interest in the way cells work can afford to limit his, or her, acquaintance with yeasts to the occasional imbibing of a well-fermented brew. Over the past decade the application of molecular cloning and related genetic techniques to that old stalwart *Saccharomyces cerevisiae* has triggered a vigorous growth of yeast research activity. Indeed these new technologies, supported by a long history of detailed fermentation and biochemical studies, now make the humble bakers' yeast a supremely versatile organism for investigation of many fundamental problems of eukaryotic cell biology. The upsurge of academic interest has been paralleled in the commercial sector, especially within biotechnology companies, by the realization that yeasts offer enormous potential as production organisms.

Yeast was launched into this ferment of activity in September 1985. It is aimed at a very distinct gap in the scientific literature — up to now, papers on yeast have been scattered throughout 20 or more journals making it difficult for anyone to keep abreast of all developments.

With the declared intention of providing "a forum for yeast researchers", the new journal includes, along with original papers and reviews, items such as news from culture collections, a calendar of meetings worldwide and reports of symposia proceedings. To date the quality of the original contributions has been high, no doubt assured by the eminence of the editorial board. Many aspects of yeast genetics, metabolic biochemistry and cell biology have been covered, although, not surprisingly, the content has been

dominated by studies on *S. cerevisiae* involving molecular biology and genetics. However, other yeasts of importance to man, such as the pathogen *Candida albicans* and the food spoilage organism *Zygosaccharomyces bailii*, have not been totally unrepresented.

The review articles are particularly helpful. They provide the reader with a concise introduction to many of the complexities of yeast biology, and they will be welcomed by both established yeast workers and newcomers. Overall, the production standards are extremely high, and the presentation is clear and inviting. This is a journal that can be read and digested comfortably in a favourite armchair.

My only criticism is of the publication frequency, which is quarterly with articles appearing six to nine months after receipt. I fear this may cause many potential contributors to continue to submit their



research papers to more frequently printed journals. For this reason, too, papers describing yeast research of major biological significance will probably continue to be attracted to the widely read, high-profile periodicals that can guarantee rapid publication.

Nevertheless if you are either an already committed yeast researcher or merely an onlooker wanting to keep pace with an exciting field, then this journal will be of interest and of service to you. Let us hope that the yeast community really does use it as its forum, by both contributing and subscribing to it. It would be regrettable if this budding journal became arrested at its start. □

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Testing the market

Bryn Bridges

Mutagenesis. Executive editors J.M. Parry and J.A. Heddle. IRL. 6/yr. \$145 (institutional), \$72 (individual).

For many years, the induction of heritable changes in cells and organisms following exposure to radiations and chemicals was studied in a rather quiet academic manner. Then, in the early 1970s, it was realized that such changes were involved in the induction of cancer, and that agents able to cause them were many and widely distributed in our environment. The result was an explosion both of fundamental work and of the testing of substances for mutagenicity.

Any new publication in this field must compete with the outstandingly successful journal *Mutation Research*, which pioneered and now dominates it and which appears in six separate sections covering every aspect and featuring every type of paper. The appearance of *Mutagenesis* does not indicate the discovery of a niche unoccupied by the major journal but rather a frank determination to compete with it. Its success will therefore be determined by the factors of the market place. Is it of better quality, is it quicker to publish, is it better value for money?

On the evidence of four early issues, the standard of design and production is high, following the pattern of its

stablemate *Carcinogenesis* which has successfully established itself in a field previously dominated by two other journals. Like *Carcinogenesis*, *Mutagenesis* has an American office and this has undoubtedly helped to establish a broad international character right from the outset. Thirty-one per cent of papers in the four issues I examined originated from Britain, 39 per cent from mainland Europe, 24 per cent from the Americas, and 6 per cent from Japan and China. Unlike *Carcinogenesis*, *Mutagenesis* is published for a learned society (the British Environmental Mutagen Society) and this has resulted in a high standard both of refereeing and of papers accepted.

In addition to original research papers, reviews and book reviews, there is a discussion forum in which controversial issues, particularly with regard to the regulatory use of mutagenicity tests, can be debated. A novel feature is the willingness of the publishers to store and make available on request laboratory data from extensive mutagenicity testing programmes, the conclusions of which are published only in summary.

It is not possible to give a realistic estimate of publication time based on the first few issues, when the receipt and publication pressures are not yet in equilibrium. If it follows the example of *Carcinogenesis*, however, the publication delay will be commendably brief.

The 1987 US subscription for *Mutagenesis* was \$150 compared with \$1,992.50 for its much larger competitor. I estimate

that *Mutagenesis* supplies nearly 4,000 words or word-equivalents per dollar, compared with less than 2,000 for *Mutation Research*.

It is already unthinkable for any worker in the field not to read *Mutagenesis* and the number of institutional subscriptions must be expected to rise sharply. Frugal librarians may then resort to a detailed consideration of whether one or more sections of *Mutation Research* could be dispensed with. It may well be that some rationalization of the latter's six sections may ensue, as well as a reappraisal of its overall competitiveness. □

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Improved access

D.C. White

Japanese Anaesthesia Journals' Review. Chief editor T. Oyama. VNU. 4/yr. 257 DM.

ANAESTHETISTS attending international conferences on their speciality will have been aware for some time of an increasing volume of scientific work coming from Japan. They will also have appreciated that the language barrier is higher and more impenetrable in the case of Japanese than it is for the European languages. This is a major obstacle to the spread of information about Japanese work and is a handicap for Japanese workers.

Japanese Anaesthesia Journals' Review attempts to improve this situation and for that reason is to be welcomed. The journal has a Japanese editorial board together with a foreign editorial board of international flavour. Each number contains summaries of about 15 papers from the Japanese literature. Each of these is a rewritten version of the original paper condensed to three or four pages of excellent English with tables and diagrams. At the end of each summary four or five references are given under the heading "Related Literature". These references are almost entirely non-Japanese and in some cases are referred to in the text and in others not. This is an unsatisfactory feature — the work summarized is deprived of its context so that its significance (or otherwise) is obscured. Perhaps it is envisaged that reading the summary will send the reader to consult the original paper. In that case it would be helpful to know whether the original is written wholly in Japanese.

The papers chosen for inclusion are said to be from a wide range of Japanese anaesthesia journals, and seven are named as being surveyed. However, of the 61 papers covered in the first volume, 49 are

Extra calcium

Iain MacIntyre

Bone & Mineral. Editor-in-chief D.V. Cohn. Elsevier. 8/yr in two volumes. Dfl. 560 plus carriage charge.

CALCIUM metabolism in its clinical and physiological aspects is a major topic at endocrine meetings in the United States; for instance, at a recent get-together of the American Endocrine Society it provided the largest single sub-group of papers, even including neuroendocrinology. The American enthusiasm for this area is due in part to Fuller Albright's pioneering eminence in calcium metabolism as well as in endocrinology, and in part to the impact on health costs now being caused by osteoporosis.

In Britain, however, calcium metabolism is usually relegated to the tail end of a Friday afternoon. It is not at all surprising, therefore, that the appearance of still another calcium journal has been met on this side of the Atlantic more with grinding of teeth than acclamation.

The new journal is supported by an

international group of scientists (International Conferences on Calcium Regulating Hormones) and has grown out of specialized conferences at first devoted only to parathyroid hormone. It is well produced and well edited, and although it hopes to cover a wider field, the important articles in the first few issues relate to clinical studies concerned with the treatment or prevention of osteoporosis.

It is rather doubtful whether the journal can carve a niche for itself in competition with *Journal of Bone & Mineral Research* and other established journals such as *Calcified Tissue*. On the evidence of the first few issues, however, it may be successful because a number of articles will be consulted by groups in the expanding field of osteoporosis, while those in the calcium field in general will probably want to read it from time to time. I expect that the journal will survive and cover a more limited field than its present attempted compass. But the next few years will decide whether that survival is more than short-term. □

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from the *Japanese Journal of Anaesthesiology*. Such a preponderance casts doubt on the editor's claim to a wide range of coverage. A partial explanation may be that the papers selected are largely from the fields of academic anaesthesiology; very little clinical work is presented.

Of the 12 papers not from the dominant journal, six are from publications not on the list of journals surveyed, and indeed I could not trace five of the listed journals in any reference source available to me. It would be of interest to see a list of all the Japanese anaesthetic journals; the existence of, for example, *The Hiroshima Journal of Anaesthesia*, now apparently in its twenty-third year, is surely unknown to most anaesthetists outside Japan.

Two final suggestions. The browser through journals in the library would be helped if the titles of papers summarized, which are printed on the back cover of the journal, were classified by subject and placed under appropriate headings such as "Respiratory", "Endocrine" and so on. Also, knowledge of the Japanese literature would be helped by provision of a list of the contents of as many as possible of the appropriate Japanese journals, together with their addresses. □

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Rhythm and reason

Steven H. Strogatz

Journal of Biological Rhythms. Editor Benjamin Rusak. Guilford Press, 72 Spring Street, New York, NY 10012. 4/yr. US \$75 (institutional), \$50 (individual); elsewhere \$90 (institutional), \$65 (individual).

THE study of cycles has always attracted more than its share of crackpots. We are told that mysterious forces wax and wane, causing stock prices to rise and hemlines to fall. Another such lunacy maintains that three separate monthly 'biorhythms' govern our mental, physical and emotional well-being.

Small wonder that the study of biological rhythms has sometimes been regarded as a fringe subject. However, over the past three decades, this young field has become respectable. Daily ('circadian') rhythms have been documented in plants and animals, from unicells to human beings, and their endogenous generation by neural and cellular oscillators is now indisputable. There have been exciting advances in understanding the molecular genetics of clocks in *Drosophila* and in *Neurospora*. The

The unwilling host

Robert J.M. Wilson

Parasitology Today. Editor C.J. Schofield. Elsevier, Cambridge, UK. 12/yr. UK Dfl.500 (institutional), £30 (individual); North America Dfl.500 (institutional), \$53 (individual).

PARASITOLOGICAL journals tend to be dull: they take a scholarly approach and have conservative formats. *Parasitology Today* can be welcomed, therefore, as a ray of light cast on this otherwise sombre scene. Resonance from such photoexcitation could reach far, because today's parasitology is by no means dull.

Recent issues of *Parasitology Today* have dealt with neurotransmitters, hormones and population genetics, as well as offering reviews on standard themes such as immunology, vaccines, and the biology of particular parasitic organisms and their vectors. Like their subjects of study, parasitological disciplines tend to feed off the parent body of mainstream science. Hence the various categories of articles in the "Review", "Focus" and "Hitech" sections are very much descriptions of applications to parasitology. Nevertheless, there has been a renaissance of interest in, for instance, diagnostic techniques (previously one of the most boring, if important, aspects of parasitology) because of the application of biotechnology to this branch of the medical and veterinary side of the subject.

With its news, book review, letter and diary sections, *Parasitology Today* can provide an important forum where diverse facets of parasitology meet; as a meeting place it should suit the many newcomers

to the field (students get a reduced subscription rate). With little injury or pain (except those inflicted on the sensibilities by a plethora of gruesome 'medical plates'), molecular biologists can muse on alternative technologies or the chemotherapeutic control of diseases which they will probably never see except in their mind's eye.

Equally, the uninformed parasitologist can grapple with the complexities of branching structures in important glycoprotein molecules, confident that years of work have been condensed to a paragraph and that there is, besides, a cartoon which gets the point over in body language. At a more aesthetic level, I noted an arrestingly successful portrayal of falciparum malaria — from *falx*, *falcis*, a scythe — shown as a skeletal figure reaping its harvest of Africa's children.

To introduce a carping note, it might be said that the punchy illustrative format of *Parasitology Today* verges on being overdone. Oversimplification and lack of substance are two obvious dangers which journals dealing in trends must guard against. If advice can be offered, it is that the core of serious, high-quality contributions should be maintained and if possible expanded. A difference both in the general level of content and restraint with illustrations is noticeable if one turns to *Immunology Today* for comparison.

The often extensive list of references allowed to reviewers, and the wide range of topics at their disposal, should enable the editors and contributors to *Parasitology Today* to fill the useful roles of disseminating both information and enlightenment whilst retaining a light touch. □

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responses of the human circadian system to bright light and phase-shifting drugs are just beginning to be worked out, offering hope for jet-lagged travellers, insomniacs and night-shift workers.

As the increasingly diverse methods of modern biology are being used to investigate such questions, it has become more and more difficult to find a common home for research on biological rhythms. Now, *Journal of Biological Rhythms* provides an excellent forum for those concerned with the temporal organization of living things.

Each issue contains about six full-length reports of original research. The editors also welcome opinion papers and reviews of timely issues, though none appeared in the first volume. Announcements of meetings and prizes occasionally appear. Papers are published within six to eight months, and are printed in an uncrowded format with large, sharply reproduced figures. Most editors and contributors are from North America.

The journal is off to a promising start. The leaders in the field are submitting some first-rate experimental research in genetics, cell biology, physiology and neuroscience. About three-quarters of the papers concern circadian rhythms, and the rest deal with photoperiodism and yearly rhythms. Topics that are currently under-represented include modelling and statistical studies, as well as medical and pharmacological aspects of biological rhythms. These are addressed more often in *Chronobiology International* (reviewed in *Nature* 323, 363; 1986) and *Chronobiologia*, although the quality of papers in the latter journals is more uneven than those in *Journal of Biological Rhythms*.

Journal of Biological Rhythms has already published several fine articles. At this rate, it may well become the leading journal in the field. □

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Inhibiting research

Paul C. Engel

Journal of Enzyme Inhibition. Editor-in-chief H.J. Smith. Harwood. 4 issues per volume (see page 372). \$270 (institutional), \$184 (individual).

FROM a purely academic standpoint, the scope announced in the title of *Journal of Enzyme Inhibition* seems remarkably narrow. Why not a journal for enzyme activation or enzyme denaturation?

The case for enzyme inhibition lies in the practical importance of the topic, especially in the design and testing of drugs and poisons. This automatically entails considerable overlap with the subject areas covered by such journals as *Biochemical Pharmacology*, *Journal of Medicinal Chemistry* and *Journal of Antibiotics*. There is, however, much to be said for a choice of journals in an active area. In this case, the declared orientation towards a type of biochemical system, rather than a single application, readily allows inclusion of general or theoretical papers that would otherwise be forced into purely biochemical journals.

Only four slim issues of *Journal of Enzyme Inhibition* have appeared over two years. This has meant a long publication delay for at least some papers, but the ultimate intention is to settle down at something approaching a quarterly publication interval.

Each issue so far contains six to eight articles. Most are full-length original papers, but there has also been a conference report on elastase inhibitors in the treatment of emphysema and a review article on renin inhibitors as potential therapeutic agents. Both are welcome features. The leading figures in a rapidly moving field are often reluctant to take time off from primary publication in order to write reviews, and a journal can perform a useful function in commissioning them. Conference reports, summarizing the current state of the debate for those unable to attend a meeting, are also valuable.

Unfortunately, the account of the Bethesda workshop on elastase inhibitors is not a good example to guide future reporters. It is curiously de-personalized, with no attribution of opinions, and no references, so that the piece does not even serve as a review. Although the pertinent issues are well aired, the newcomer is offered no easy entry to the field. This, however, is not a criticism of the journal's basic intention to include such reports. The elastase report had already appeared elsewhere, and perhaps the editors of the new journal will take a firmer line with articles they have specifically commissioned to ensure that they

faithfully convey the flavour of active discussion.

Despite the slow start, the journal has attracted papers by a number of well-respected workers, and has covered a commendably wide range of topics. On the one hand there are papers of obvious pharmacological interest on renin, GABA transaminase, acetyl cholinesterase, NADPH-cytochrome P450 reductase and so on, but equally there are papers on the interaction of alkaloids with yeast alcohol dehydrogenase, on mechanism-based inactivation of yeast pyruvate decarboxylase, and on the kinetics of modification-induced protein unfolding.

In all, this journal should interest a

substantial body of readers involved in pharmaceutical and toxicological aspects of enzymology. For them it will serve a useful function by drawing together both the practical and the more theoretical aspects of enzyme inhibition.

One final technical point: although the inclusion of colour plates for histochemical illustrations is very welcome, the quality of the half-tones is disappointing. The ordinary paper used for the text is not really of good enough quality for photographs, and it is pointless to show a stereo model of an enzyme (renin) unless it is well reproduced. □

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An insight into infection

Clive Sweet

Microbial Pathogenesis. Editor-in-chief Helena Mäkelä. Academic. 12/yr. UK £135 (institutional), £52 (individual); North America \$248 (institutional), \$78 (individual).

Microbial Pathogenesis was launched by Academic Press to provide a focus for publication of research on the molecular and cellular biology of infectious disease. The appearance of the journal is timely, because the rapid advances in molecular biology are being applied with much success to infectious disease; also, it fills a niche that other journals have attempted to accommodate by inclusion of sections devoted to microbial pathogenesis.

While the existing journals essentially cater for specific groups of pathogens — for example viruses in the *Journal of Virology* and bacteria in *Infection and Immunity* — *Microbial Pathogenesis* covers all pathogens, from viruses to parasites, because it recognizes that there are features common to infections produced by different kinds of agents and hence the emergence of pathogenicity as an identifiable research area. As yet, contributions on bacterial pathogenesis outnumber those on viral pathogenesis by about three to one, and only the occasional paper has appeared on yeast and protozoa, possibly reflecting the relative activities in these areas of research.

Studies of pathogenesis also require a multi-disciplinary approach, drawing upon pathology, microbiology, immunology, physiology, biochemistry and genetics. All of them are encompassed within this one publication.

The journal has an international character, with an editorial board representing ten countries, although Europe and North

America predominate. Similarly, few papers from outside Europe and North America have yet appeared. The speed of publication is reasonably rapid — generally within six months of the acceptance date — but there may be up to a further four months additional delay from submission to acceptance largely occurring when revisions are necessary. That the latter are frequently required testifies to the intention of the editors and their editorial board to establish a high standard for the journal, which so far it has attained. The quality and standard of production is also high, with clear tables, line drawings and half-tone figures. Short communications are accepted but they are not rapid publications. Occasional mini-reviews and book

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reviews are also included. Fifty free reprints are offered and there are no page charges.

A journal dedicated to this area, but independent of the rather dominant and well known American journals, must be a good thing for authors outside the United States who often feel aggrieved by the high page charges and occasional xenophobia among referees. That the journal does meet a need is evidenced by an increase from six to twelve issues per year. This has inevitably reduced the number of articles from an average of ten per issue to eight per issue, and the number of pages from an average of 99 to 79 per issue.

Although *Microbial Pathogenesis* might appear to be merely yet another new journal in an area not without existing coverage, I feel that it deserves to succeed. Because of its aims, appositeness and quality, its future should be assured. □

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